DECLARATION

I hereby declare that this submission is my own work towards the 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 acknowledge has been made in the text.

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DEDICATION

This work is dedicated to my husband, Mr Maxwell Osei Brown.

ACKNOWLEDGEMENT

I am most grateful to the Almighty God for his abundant mercies throughout my entire study. I would like to express my most sincere and heartfelt gratitude to my supervisor Professor R.C. Abaidoo, for his insightful guidance, invaluable patience and steadfast encouragement during the preparation of this thesis. I also wish to register my profound gratitude and appreciation to my field instructors, Dr. Bernard Keraita and Dr. Philip Amoah, all of International Water Management Institute (IWMI) for the support and cooperation offered during the fieldwork. I am also grateful to have had the encouragement and support of many friends and fellow graduate students during this process: Maxwell Akple, Grace Anso Ziem, and Cordelia Dawson Kesson for their time and input in making this work a success. It is indeed a great joy to work with people of such overwhelming temperament. Finally, my appreciation goes to my husband, Mr. Maxwell Osei Brown and the entire Owusu family for the unwavering support all these years.

ABSTRACT

In Ghana, urban sanitation infrastructure is poor and only a small portion of the wastewater generated is collected for treatment. The bulk ends up in drains and nearby water bodies and is used by vegetable farmers for irrigation. The purpose of this study was to assess the effectiveness of using locally feasible non-treatment interventions such as good farming and handling practices through producer-consumer pathway to reduce contamination on wastewater-irrigated spring onions. Spring onion samples were collected between November and April, 2008 in the dry season and analysed for thermotolerant coliforms by the MPN (three tubes) technique and helminth eggs using the flotation sedimentation method. The Statistical Package for Social Scientists (SPSS) version 13.0 was used for testing the various statistical relationships between and within variables. The initial study tested the effectiveness of cessation of irrigation prior to harvesting, management of watering can irrigation to reduce contamination and determination of major source of contamination of spring onions at production site. Post-harvest feasibility study was also carried out to understand the current handling practices of spring onions by market women, which served as a guide to develop appropriate ones. Sanitizers (salt and vinegar solution) were also tested for their efficacy in decontaminating spring onions at the kitchen. Spring onion was tracked from farm, to the market, and to the kitchen and subjected to selected feasible interventions. Samples were taken before and after each non-treatment intervention treatment and their effectiveness assessed. The result of the study showed that, an average of 0.52 log units for thermotolerant coliforms and 0.06 helminth eggs per 100 g of spring onions were

removed on each day of cessation of irrigation. This corresponded to an average daily loss of 0.15 kg fresh weight of spring onions. Decreasing watering heights, whether with watering cans perforated or not increased thermotolerant coliforms significantly since results showed that bulbs of spring onion harbou6red the major source of contamination. Survival of thermotolerant coliforms and helminth eggs was higher in spring onions stored in sacks and lower in those stored in baskets. At the market, washing of spring onion (whole plant) with water proved to be the best option for reducing both thermotolerant coliforms and helminth eggs. However, washing affected the firmness of spring onion leaves, and as a result washing only the bulbs in a bowl of water (8.5 litres) for two minutes, was selected as the easily adoptable intervention. Use of vinegar solution was a more effective sanitizer than salt solution at the kitchen. The sum of the individual log unit reduction for the different non treatment options assessed in the study equal to 5.07 (vinegar) and 5.0 (salt solution) log units for thermotolerant coliforms with 2.2 (vinegar) and 1.5 (salt solution) helminth eggs for vinegar and salt solution, respectively. The multiple barrier approach (tracking same stock of spring onions from farm to kitchen) study suggests that to prevent thermotolerant coliforms and helminth eggs contamination on spring onions, adequate pre-harvest protection against pathogen contamination and post harvest cleaning and disinfection strategies need to be employed.

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ACRONYMS

ANOVA	Analysis of variance
APHA	American Public Health Association
FAO	Food and Agriculture Organization
FDA	Food Disinfection Agency
GDP	Gross Domestic Product
H_2SO_4	Sulphuric acid
HOCl	Hypochlorite acid
IWMI	International Water Management Institute
ICMSF	International Commission on Microbiological Specification for food
KNUST	Kwame Nkrumah University of Science and Technology
MPN	Most Probable Number
NACMCF	National Advisory Committee on Microbial Criteria for Foods
Ppm	Parts per million
NPK	Nitrogen, Phosphorus, and Potassium
Rpm	Revolution per minute
SPSS	Statistical Package for Social Scientist
UNDP	United Nations Development Programme
UPA	Urban and Peri-urban Agriculture
US-EPA	United State- Environmental Protection Agency
VTEC	Verotoxin-producing *Escherichia coli*
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

There has been an increasing interest in reuse of wastewater in agriculture over the last few decades due to increased demand for fresh water (Blumenthal and Peasey, 2000a). Population growth, increased per capita use of water, the demands of industry and of the agricultural sector, all put pressure on water resources. A major mechanism that can be used to achieve greater efficiencies is the reuse of water that once would have been discarded into the environment after use (Toze, 2006). The reuse of wastewater has been successful for irrigation of a wide array of crops and increases in crop yields from 10-30% have been reported (Asano and Levine, 1998). Vegetable farming, which has high water requirements, is mainly dependent on irrigation. It is therefore mainly practiced on valley bottoms along streams, which are now practically wastewater conduits. Farmers do not pay for this water and they perceive the nutrients in the wastewater and its year round availability as advantages. Nevertheless, these water sources are known to be contaminated and in most cases contamination levels significantly exceed the WHO guidelines (Keraita *et al*., 2003).

Many West African studies have reported high levels of pathogen contamination on vegetables at the farm, market, and kitchen level (Cisse, 1997; Olayemi, 1997; Armar-Klemesu *et al.*, 1998; Faruqui *et al.*, 2004) which far exceed International standards. In recent years, the frequency of epidemics associated with vegetables have increased in some industrialized countries as a result of change in dietary habits and increased import

of food (Altekruse *et al.*, 1997), and use of marginal water for vegetable production. However, in developing countries with poor sanitary conditions and a larger range of risk factors, many foods related outbreaks probably remain undetected (Beuchat, 1998). In Ghana, agriculture contributes 36.6% of the gross domestic product (GDP) and employs 60% of the labour force. The average annual *per capita* income of those employed in agriculture is estimated at US$390. It is estimated that 60% of some 10000 ha of irrigated fields in Ghana, are irrigated with wastewater (Agodzo, 1998; Obuobie *et al.*, 2006). Irrigated agriculture is therefore important in producing about 90% of vegetables consumed in the cities and providing a major source of income for the households (Drechsel *et al.*, 2002). The growing demand for fresh and perishable agricultural produce in the major cities drives the all year round urban and peri-urban vegetable production. The crops that are frequently irrigated with wastewater include, cabbage, lettuce, spring onions, tomatoes, and carrots which are often used in exotic diets and frequently eaten raw or with low heat application.

1.2 Problem statement

Wastewater makes up an important resource for intensive agricultural production by urban and rural poor and thereby strengthens their livelihood opportunities. Agricultural produce cultivated with wastewater adds importantly to the food security of poor rural and urban communities. Yet, the potential transmission of wastewater-associated diseases is high in farmers and consumers. Irrigation of salad crops eaten uncooked with untreated wastewater can result in the transmission of intestinal nematode infections and bacterial infections (WHO 1989). In Ghana, risk assessments done in Kumasi, Tamale and Accra

show high faecal contamination levels in irrigation water and vegetables (3-8 log units) in irrigated urban vegetable farms (Amoah *et al*., 2005, 2006; Obiri-Danso *et al*., 2005).

The WHO standard for intestinal nematodes (helminth eggs) is ≤1 egg / litre and faecal coliforms in irrigation water is ≤1000 faecal coliform/100 ml and serve as an irrigation water quality standard (Hespanhol and Prost, 1994). To achieve this, WHO in 1989 developed guidelines for wastewater use with greater emphasis on wastewater treatment. The application of these guidelines posed many difficulties for less endowed countries especially in relation to urban agriculture. For instance, in many low-income countries, like Ghana, wastewater treatment as expected by the guidelines is not possible due to a variety of (mostly economic) reasons. The provision of sanitation infrastructure has not kept pace with population growth rates, leading to increasing volumes of wastewater being discharged directly to the environment. For efficient collection and treatment of wastewater, much investment is needed, which is not readily available.

In Ghana, statistics have shown that 63% of the population have access to improved sanitation facilities, but the distribution is poor with more than 70% of the population in the northern part of Ghana having no access to sanitation facilities (www.ruaf.org/system). Of the 42 sanitation plants in Ghana, more than half are in Accra and most of them, especially the public ones with larger capacity, are not or only partially functional. Based on these statistics, it can be inferred that treatment of wastewater for irrigation purposes is not a dependable and sustainable option for Ghana. Therefore, enforcement of the WHO guidelines in such situations would stop hundreds or thousands of farmers from irrigating their vegetable with water from polluted streams and put their livelihoods at risk. Restrictions would also negatively affect food traders and the general

market supply with these vegetables, especially in cases where other water sources are inaccessible. As a result of these difficulties, it was suggested during an Experts consultation meeting in Hyderabad, India, in November 2002 that the WHO guidelines needed adjustment for better applications in wastewater use in urban and peri-urban agriculture especially in poor countries.

The overall goal of the revision was to find a better balance between safeguarding consumers' (and farmers') health and safeguarding farmers' livelihoods. The new WHO guidelines (WHO, 2006) are more flexible and develop further the concept, which considers treatment as only one component of an integrated risk management strategy. In view of this, WHO (2006) proposes the concept of multiple barrier approach. There are multiple barriers that reduce exposure of pathogens to the different target groups, the farmers, the nearby communities, and the consumers. These barriers include cessation of irrigation prior to harvesting to enhance pathogen die off, use of application techniques that reduce produce contamination, prevention of cross-contamination, improved food hygiene, better cooking of food, reducing crop contamination by washing, disinfecting, peeling, etc. The barriers can also be referred to as non-treatment interventions as they do not require the use of high-energy technologies. This multiple barrier approach involves a combination of non-treatment interventions from farm to fork thereby interrupting the flow of and recontamination by pathogens. The intervention approaches will therefore have to be locally appropriate so that farmers and others can easily adopt them in the food chain to reduce potential health risks (Drechsel *et al*., 2006).

Studies conducted in Ghana have assessed some non-treatment interventions at farm and kitchen level on lettuce and cabbage (Keraita *et al*., 2007a; Amoah *et al*., 2007)

but did not include spring onion which is also a wastewater irrigated vegetable and often eaten raw. Preliminary results from the study indicated significant reduction in microbiological contamination of these wastewater irrigated vegetables. This study intends to bridge this knowledge gap by using spring onion as the test crop to assess the effectiveness of some non treatment interventions from the farm to the kitchen. This has become necessary as its patronage in our local markets for home consumption is increasing rapidly.

1.3 Research questions

1. Does cessation of irrigation and the use of watering cans at different heights on-farm reduce thermotolerant coliforms and helminth eggs numbers on spring onions significantly;
2. Does a combination of interventions (e.g. storage in basket and washing the bulb of spring onions, and displaying in baskets at market) significantly reduce thermotolerant coliforms and helminth egg numbers;
3. Does washing of spring onions with salt solution or vinegar at the kitchen reduce thermotolerant coliforms and helminth egg numbers significantly; and,
4. Do spring onions produced from two-days cessation of irrigation prior to harvesting, storage in baskets, washing of bulbs in a bowl of water and display in basket, and washing in salt or vinegar solution during production, sale in the market and handling in the kitchen significantly reduce thermotolerant coliforms and helminth egg numbers?

1.4 Objectives of study

This study sets out to assess the effectiveness of a combination of non-treatment interventions in reducing microbial health risks associated with wastewater irrigated spring onion.

Specifically, the study was to:

1. assess the effectiveness of cessation of irrigation prior to harvesting and use of watering can in reducing the numbers of thermotolerant coliforms and helminth egg numbers on spring onions on-farm;
2. identify feasible post harvest handling practices that can reduce thermotolerant coliforms and helminth egg numbers on spring onions at the market;
3. assess the effectiveness of salt or vinegar solution use at the kitchen in reducing thermotolerant coliforms and helminth egg numbers on spring onions; and,
4. assess the effectiveness of combining two-days cessation of irrigation prior to harvesting on farm, storage of spring onions in baskets, washing of bulbs in a bowl of water and display in basket at the market, and washing in salt or vinegar solution in reducing thermotolerant coliforms and helminth egg numbers on spring onions, along the production consumption pathway.

CHAPTER TWO

LITERATURE REVIEW

2.1 Driving forces behind increasing wastewater use

The use of urban wastewater in agriculture is centuries old practice that is receiving renewed attention with the increasing scarcity of fresh water resources in many arid and semi-arid regions (Scott *et al.,* 2004). Driven by rapid urbanization and growing wastewater volumes, wastewater is widely used as a low-cost alternative to conventional irrigation water; it supports livelihoods and generates considerable value in urban and peri-urban agriculture despite the health and environmental risks associated with this practice. The use of wastewater for crop irrigation reduces the use of artificial fertilizers and is thus an important form of nutrient recycling. Thus supplementary fertilization needs can be reduced (or even eliminated) for some crops, with a subsequent increase in farmers' net income. Additionally, efficient use of nutrients in wastewater reduces the environmental impacts associated with the production of mineral fertilizers.

2.2 Worldwide practice of wastewater irrigation

Wastewater is increasingly reused worldwide. UNPD (2000) estimated that about 800 million people are engaged in urban and peri-urban agriculture (UPA) worldwide and contribute about 30% to the world's food supply. At least 10% of the world's population

is thought to consume foods produced by irrigation with wastewater (Smith and Nasr, 1992). These probably explain why there are many successful wastewater use schemes throughout the world where nutrient recycling is a major benefit (Pescod and Arar, 1988; FAO, 1992).

Recent surveys across 50 cities in Asia, Africa and Latin America show that wastewater irrigation is a common reality in three-fourths of the cities. In Vietnam and Pakistan alone, between 10,000 and 30,000 hectares are cultivated with undiluted wastewater. The Mezquital valley in Mexico, which is probably the largest irrigation area using raw wastewater, covers more than 90,000 hectares. Mexico accounts for about half of the 500,000 hectares irrigated with wastewater in Latin America. Global estimates of the total area under raw and diluted wastewater irrigation are still fragmentary, but might range from around 3 to 3.5 million hectares, with the largest share probably in China. This is twice the area under formal vegetable irrigation in the whole of Africa.

2.3 Irrigated urban agriculture in Ghana

In Ghana, urban agriculture is mainly characterized by backyards and commercial small-scale irrigated vegetable farming. The main source of irrigation water is obtained from urban streams and drains. Most urban centres have no means of treating wastewater and only 4.5% of households in Ghana are connected to sewer networks (Ghana Statistical Services, 2002). This leaves most untreated wastewater, mainly from domestic sources, ending up in urban drains and water bodies in and downstream of the cities. This water forms reliable sources of irrigation water for urban vegetable farmers in Ghana allowing them to grow perishable vegetables all-year-round. It is estimated that 60% of some

10000 ha of irrigated fields in Ghana are irrigated with wastewater (Agodzo, 1998, Obuobie e*t al.*, 2006). Sonou (2001) conducted interviews with farmers in Accra about the source of irrigation water and marketing of irrigated vegetables and as many as 60% confirmed using wastewater, with 23%, pipe-borne water, and 17% using piped water stored in a ground reservoir. These percentage differences are a clear indication of the important role wastewater plays in urban agriculture in Ghana.

2.4 Irrigated urban agriculture in Kumasi

Kumasi has a semi humid, tropical climate with a total average rainfall of 1340 mm. approximately 90% of the annual total falls between March and October. Natural drainage runs from north to south. About 500 urban farmers are involved in 'bottomland' vegetable farming throughout the year, with up to 15,000 farmers cultivating in the peri-urban areas during the dry season. While most farmers, especially in urban Kumasi, are men, women dominate the marketing. With an estimated area of 11,900 ha under dry season vegetable farming around Kumasi, revenue generation from irrigation has been estimated from farm surveys to be as high as US $6 million (US $500/ha/yr) with profits of at least US $4 million (Cornish and Lawrence, 2001).

In Kumasi, polluted rivers and streams are the main sources of water for 70% of the farmers. There is an extensive use of shallow dug wells (27%) on valley bottoms especially in the urban area. Out of seventy farmers interviewed in a survey conducted by International Water Management Institute (IWMI) in Kumasi, 75% of the farmers used wastewater as the source of irrigation water because it is accessible and reliable. Piped water is not only expensive but is unreliable and, in any case, inaccessible to most

farmers (Obuobie *et al*., 2006). Irrigation takes place in the morning and evening. Due to the short growing cycle of many vegetables and their fragile nature (loss of attractive appearance), irrigation continues until harvesting day. In urban Kumasi, farmers grow non-traditional vegetables like lettuce, cabbage, and spring onions on open spaces, with water access throughout the year. They rely on this as their major source of income. The kind of vegetables grown depends mostly on actual market demand, water availability and farmers' specialisation and experience with their cultivation and management. Many farmers combine two to three crops in a growing season and reports of many harvests per year especially for lettuce and spring onions, are not uncommon.

2.5 Spring onions

Onions are from the *Allium* family, which includes leeks, shallots, spring onions, chives and garlic. Most of what are sold as spring or salad onions are simply immature or early-maturing varieties of onion. The spring onion are onions which probably started out as the spring thinning from ordinary onions. Spring onions are sub-divided into two main parts (bulbs and leaves). The bulbs are embedded in the soil while the leaves stick out on the soil surface. They are onions that are harvested early with the bulbs still small and the leaves still green. They have a small bulb connected to a white stem. The straight, hollow leaves spring from this white stem. Both the white stem (bulbs) and leaves are the main edible part of the plant and is used commonly in salads. The sharp-sweet, aromatic flavour of the leaves is especially appreciated in cooking, where they are used raw in various salads or as delicious filling in pies. Spring onions are good sources of vitamins B and C, folate and fibre. They are relatively high in flavonoids, an antioxidant that is

thought to protect against cancer and heart disease. Spring onions are notoriously sandy and must be washed thoroughly before consumption. According to Blumenthal *et al.* (2003), certain crops may be more susceptible to contamination than others and an example is onions, which are root crops. Most commercially cultivated onions are grown from the plant's small black seed, which is sown directly in the field, but onions may also be grown from small bulbs (or offsets) from the mother bulbs.

Onions can be grown in almost any type of soil but most members of the family like a friable, open, slightly sandy soil. All members of the onion family prefer an open, sunny position; they do not grow well under the shade of trees or buildings, nor do they like the competition of roots from trees or large shrubs. Because of their small root systems they will need to be kept fairly moist in order to reach their water supply requirements. They are quick to grow and should be ready for harvesting in around eight to twelve weeks when the leaves are 30-to-40 cm high. Onions are heavy feeders and require a considerable amount of fertilizer, particularly nitrogen.

2.6 Sources of municipal and industrial effluents in Kumasi

Salifu and Mumuni (1998) reported that the Kumasi metropolitan area has sewerage for less than 4% of the residents. Forty percent of the residents depend on public toilets (improved pit latrines, aqua privies, and pan latrines); 15% depend on septic tanks (without soakaways), less than 10% have household improved pit latrines and 35% use the free range areas such as bushy areas, refuse dumps and along river banks (Ghana Statistical Services, 2002).

Between 250 and 350 m^3 of sewage and night soil are collected daily and by vault emptying trucks. Until recently, this material was discharged into poorly maintained waste stabilization ponds. Retention time was very short and ineffectively treated sewage pass directly into the Subin River. Even with effective waste stabilization ponds in place, much of the domestic sewage and industrial effluent from Kumasi continue to be discharged directly into streams passing through the city. The major sources of industrial effluent in the city are two breweries, soft drinks bottling plant, and a soap factory. Light industrial activities at the Suame Magazine complex-draining to the northwest and sawdust mounds at sawmills also generate significant amounts of waste oil and leachate, respectively. The untreated wastewater flows through channels into rivers where it is diverted by subsistence farmers to small plots where unregulated vegetables are grown for nearby urban markets (Shuval *et al*., 1990).

2.7 Sources of contamination of vegetables

When vegetables are consumed raw, as is the case with salads, harmful microorganisms that may be present are ingested. Traditionally, eating raw fresh fruits and vegetables from the field was considered safe; however, bacterial pathogens are currently being found in or on fruits and vegetables (Ackers *et al*., 1998; Beuchat, 1996; De Roever, 1998; Mead *et al*., 1999). Contamination event may occur either during preharvest, harvest, or postharvest. Most of the contaminating flora is non-pathogenic and has a natural occurrence on the produce. However, pathogens from the human and animal reservoir as well as other pathogens from the environment can be found on the vegetables.

2.7.1 Pre-harvest contamination

2.7.1.1 Presence of pathogens in soil amendments and irrigation water

Sources of microbial pathogens on fresh produce at the preharvest stage include faeces, irrigation water, inadequately composted manure, soil, air, animals, and human handling (Beuchat, 1996; Buck *et al*., 2003). Animal manure is frequently used as fertilizer and soil conditioner. Adding manure to the soil has agronomic benefits through the addition of plant nutrients (nitrogen, phosphorus, and potassium) and organic matter Manure nutrients help build and maintain soil fertility. Manure can also improve soil tilth, increase water-holding capacity, lessen wind and water erosion, improves aeration, and promotes growth of beneficial organisms (Gagliardi and Karns, 2002). However, animal manures frequently contain enteric pathogenic microorganisms (Pell, 1997) and land spreading of manure can lead to pathogen entry to the food chain. Cross-contamination of produce with manure or improperly composted manure used on the farm can be a source of pathogen during preharvest. Although competition with soil microorganisms and adverse environmental conditions can reduce pathogen populations, there is little information regarding the degree to which these pathogens can survive in manure-amended soils and also on vegetables that are grown on those soils.

In Kumasi, the use of poultry manure is very common due to its high availability and low price (US $0.1 per 50 kg). Only a few farmers use mineral fertiliser in addition, (mostly for cabbage production). In urban Kumasi, many more vegetable farmers use mineral fertilisers (US $14 per 50 kg NPK) but combine it with poultry manure when possible (Danso and Drechsel, 2003). Several surveys have been carried out to determine

the presence of pathogens in various forms of animal wastes destined for application to crop land. In Belgium and Finland, *Listeria monocytes* were found in 6.7 to 20% of poultry manure analysed (Husu, 1990; Van Renterghem *et al.*, 1991). Vernozy-Rozand *et al.* (2002) also conducted a study to determine the presence of verotoxin-producing *Escherichia coli* (VTEC) in manure, slurries and composts in France. The strains identified were potentially pathogenic for humans and further emphasized the need for appropriate handling and use of manure, slurry, and compost so that the risk of contamination of fruits and vegetables VTEC could be minimized.

Water is mainly used for irrigation of plants and its quality varies depending on whether it is surface water or potable water. A recent study of two sites in the Accra Metropolitan Area (Sonou, 2001) revealed that wastewater was the most frequently used water for irrigation purposes. As much as 60% of the farmers interviewed at Dzorwulu Power Pool Station and at Castle Parks and Gardens (32.3%) confirmed the use of this type of water; less than a quarter (23.3%) use pipe-borne water while approximately 17% use piped water stored in a ground reservoir. Surface water from streams and lakes used for irrigation may be contaminated with pathogenic protozoa, bacteria, parasites, and viruses. The survival of most pathogens, once discharged into a water body, is highly variable depending upon quality of the receiving water, particularly turbidity, oxygen levels, presence of pesticides and nutrients, temperature and solar radiation (Moore *et al.*, 1998). The risk of water-borne infection from any of these pathogens can be reliant on a range of factors including pathogen numbers and dispersion in water, the infective dose required and the susceptibility of an exposed population, the chance of faecal contamination of the water and amount of treatment undertaken before potential exposure

to the water (Haas *et al*., 1999). Four groups of pathogenic microorganisms have been identified to be potentially present in wastewater; these are bacteria, helminths, viruses, and protozoa. Bacteria (thermotolerant coliforms) are used as an indicator of faecal contamination as they are easily detectable and found in high numbers in the faeces of warm-blooded animals (WHO, 1996). Helminth eggs also have wide varying persistence in the environment (WHO, 2006). To this end, the main monitoring organisms for the most recent WHO guideline for wastewater reuse are thermotolerant coliforms and helminth eggs (WHO, 2006).

2.7.1.2 Bacteria

Pathogenic or potentially pathogenic bacteria are normally absent from a healthy intestine unless infection occurs. When infection occurs, large numbers of pathogenic bacteria will be passed in the faeces thus allowing the spread of infection to others. Many bacterial populations decline exponentially so that 90 to 99% of the bacteria are lost relatively quickly. Survival of bacteria, like many other organisms, depends greatly on how hostile the environment is. There are many different types of disease-causing bacteria, and they are usually present in low numbers, which do not always show up in tests. Thermotolerant coliforms are present in higher numbers than individual types of pathogenic bacteria, which can be tested for relatively easily. Thermotolerant coliforms are group of bacteria whose presence in the environment usually indicate faecal contamination; previously called faecal coliforms. They are distinguished from total coliforms by their ability to tolerate elevated incubation temperatures during culturing. Thermotolerant coliforms include the portion of the total coliforms group capable of

forming gas within 24 hours at 44.5°C (APHA, 1998). This group includes members of the genera *Escherichia, Klebsiella, Enterobacter, and Citrobacter*.

2.7.1.3 Helminths

Helminths are parasitic worms that belong to three biological categories: nematodes (round worms), trematodes (flukes) and cestodes (tapeworms). The severity of helminth infection depends on the number of worms, which have invaded the body from outside. This worm load also determines the rate at which infection is propagated by transmission of eggs in the faeces, urine or sputum of the human host (Pacey, 1998). Aquatic nematodes are the major helminths associated with wastewater irrigation and their success is attributed to their ability to resist chemicals, which are instantly fatal to other organisms. Helminths are more persistent in harsh environment and thus are good index organism for the assessment of health risks associated with wastewater reuse in developing countries (Hamilton *et al*., 2006). Most helminths are found in natural waters as a result of discharge of effluent, activated sludge, sewage, excreta and faeces from cattle, rodents, man, etc. Some of the helminths infect man through ingestion of contaminated vegetables. Examples of such helminths are *Ascaris spp*. and *Hymenolepis spp*. Some also, infect man through direct contact by irrigation workers, with skin (e.g. barefoot) exposed to the wastewater.

In order to understand the role of wastewater in the transmission of helminth infection, it is appropriate to consider the various means, which parasites employ to ensure their survival and spread. There are four stages of transmission of helminths eggs which can be looked at: (a) escape of eggs or larvae into the environment, (b)

development and survival in the environment (sometimes in another animal referred to as intermediate host) (c) the infection of another human host and (d) the adult live within the body, where the eggs are produced to restart the life cycle as illustrated in Figure 2.1. If any of the stages can be effectively blocked, the continuous transmission of the parasite can be interrupted and human infestation reduced to lower levels or may even disappear.

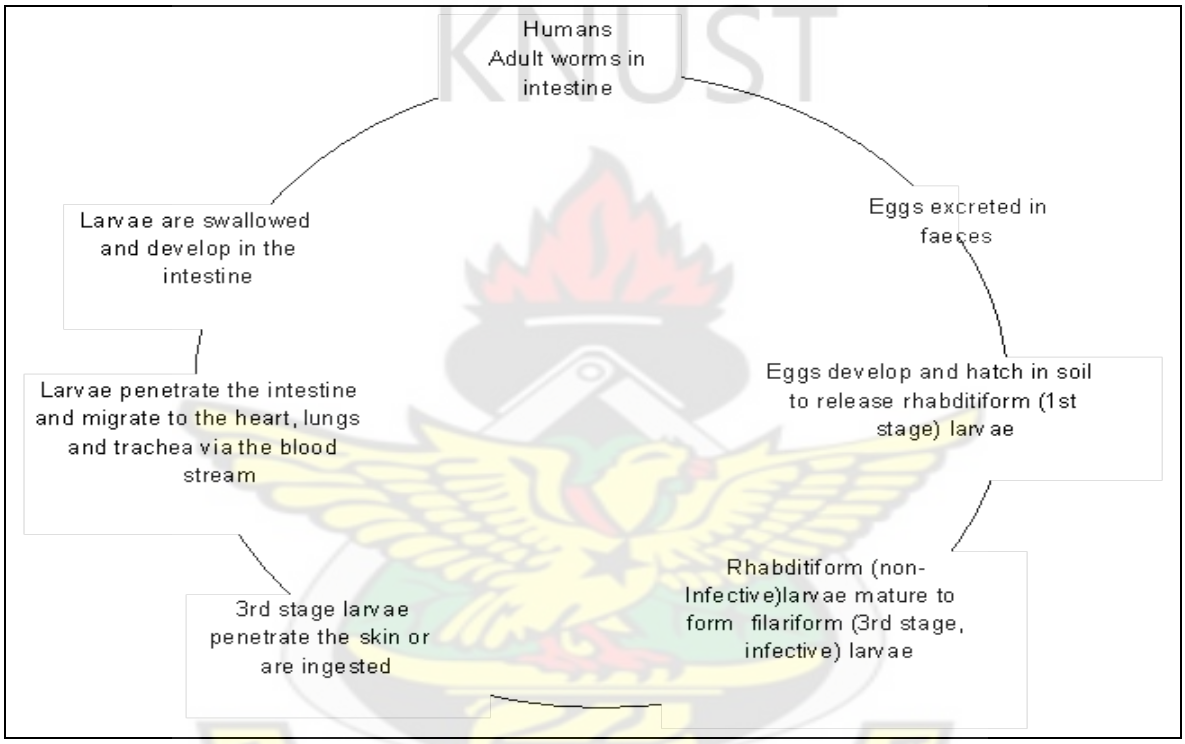

Figure 1: Typical pattern of helminth infection cycle

Source: (www.soton.ac.uk/-ceb/Diagnosis/vol 2.htm

Helminths are endemic in many areas and are associated with poor hygienic practices. Different types of helminths including *Ascaris lumbricoides* have been isolated from wastewater-irrigated vegetables sold in the market of Ghana (Obuobie *et al.*, 2006). *Ascaris* (roundworms) is one of the most resistant of the enteric pathogens and it is often used as a parasitological indicator (Watson *et al.*, 1999). About 25% of the world

population is infested with *Ascaris* and the infection is more prevalent in the developing world (Blumenthal *et al.*, 1989).

Table 1: Excreted organism concentrations in wastewater

Organism	Numbers in wastewater (per litre)
Bacteria	
Thermotolerant coliforms	10^8–10^{10}
Campylobacter jejuni	10–10^4
Salmonella spp.	1–10^5
Shigella spp.	10–10^4
Vibrio cholera	10^2–10^5
Helminths	
Ascaris lumbricoides	1–10^3
Ancylostoma/Necator	1 - 10^3
Trichuris trichiura	1- 10^2
Schistosoma mansoni	ND
Protozoa	
Cryptosporidium parvum	1–10^4
Entamoeba histolytica	1–10^2
Giardia intestinalis	10^2–10^5
Viruses	
Enteric viruses	10^5–10^6
Rotavirus	10^2–10^5

ND, no data
Source: Feachem *et al.* (1998)

Failure to adhere to hygienic standards in the kitchens of institutions like schools, hospitals, restaurants and hotels can lead to the widespread of helminthic infections caused by *Ascaris lumbricoides,* hookworms, *Enterobius vermicularis, Trichuris spp. Toxocara spp*., and Trichostrongylidae to humans as a result of consumption of improperly washed vegetables and fresh fruits used as salad ingredients (Coelho *et al*., 2001).

2.7.1.4 Survival of pathogens on vegetables

Pathogens survive on crop surfaces for a shorter time than in the soil as they are less well protected from the harsh effects of sunlight and desiccation (Moore *et al.*, 2006). Survival and growth of pathogens on vegetables depends on many factors, one of which is whether the pathogen is able to attach to the surface. The attachment of the pathogen to the surface of the vegetable is governed by number of factors including temperature, pH of the vegetables, and water activity (Iturriaga *et al*., 2003a). Plant pathogenic bacteria attach to the surface through both reversible and irreversible attachments, which involves weak van der Waal force of attraction between the cells and the surface (Iturriaga *et al.*, 2003b).

It has been reported that adsorption of the micro-organism to the surfaces is related to hydrophobicity of the bacterial strain and it was said that the least hydrophobic bacteria show the least adsorption (Burnett *et al*., 2000). The degree of attachment has practical implications. Attached bacteria are difficult to wash off and it is generally accepted that approximately 10% of enteropathogens are not removed by washing.

After attachment of the pathogen onto the vegetable, there is internalization, which takes place through the stomata, lenticels, and punctures on the surface (Reina *et al*., 2002). Various internalization studies have been done in the past to show the possible pathway of the bacteria into the plant cell and bioluminescent studies have shown that the bacteria could colonize early in the roots of the germinating seedlings. It has been reported that the bacteria can enter the plant cell through any crack in the epidermis and through the fissures that are formed due to lateral roots (Warriner *et al*., 2003). A variety of pathogenic bacteria has been isolated from vegetables and fruits (Beuchat, 2002);

some of these bacteria are *Salmonella, Shigella, Vibrio cholerae,* and *Clostridium botulinum.* Surfaces of contact are also known to affect the survival of the pathogens and studies show that as compared to free cell suspension, salmonella exhibit more resistant to antimicrobial agents and temperature abuse when the bacteria are attached to surfaces (Dhir and Dodd, 1995).

Most pathogens have the tendency to stick to a surface, not only because it offers them a protective microenvironment, but also a nutritional advantage. Studies of microbial colonization of surfaces have shown that most pathogens grow on surfaces enclosed in biofilms. These are encased microcolonies of bacterial cells attached to vegetable surfaces by way of adhesive polysaccharides excreted by the cells. This formation greatly affects the rate of microbial metabolism and thus enhances survival of pathogens on vegetable surfaces.

2.7.1.5 Die-off or survival of excreted pathogens

The die-off of excreted pathogens is an important factor influencing transmission. In principle, all pathogens die-off upon excretion. Prominent exceptions are pathogens whose intermediate stages multiply in intermediate hosts as the miracidia of *Schistosoma* which multiply in aquatic snails and are later released into water body. Some bacteria such as *Salmonella*, *Shigella*, and *Campylobacter*, have the potential to multiply outside the host predominantly on food and at warm temperature. The pathogens have resistance, and worms are among the more resistant with *Ascaris* surviving the longest especially in the soil (Table 2.2). The main factors influencing die-off are temperature, dryness, and UV-light. Die-off rates increase in proportion to the level or intensity of these variables.

Table 2: Survival times of selected excreted pathogens (helminths and bacteria) in soil and on crop surfaces at 20-30°C

Organism	Survival times (days)	
Helminths	In soil	On crops
Ascaris lumbricoides eggs	Years	<60 but usually <30
Hookworm larvae	<90 but usually >30 days	<30 usually <10
Taenia saginata eggs	Many months	<60 usually < 30
Trichuris trichiura eggs	Many months	<60 usually <30
Bacteria		
Thermotolerant coliforms	<30 usually <15	<60 usually <30

Source: WHO (1989) as summarised by Feachem *et al.* (1998)

2.8 Harvest

Vegetables can become contaminated with pathogenic microorganisms during harvesting through the presence of faecal material, human handling, harvesting equipment, transport containers, contact with wild and domestic animals, air, transport vehicles, ice or water (Beuchat, 1995). In an investigation of several foodborne illnesses associated with fresh produce, agricultural workers were in many cases the likely source of the pathogen (National Advisory Committee on Microbial Criteria for Foods, NACMCF, 1999). Lack of suitable sanitary hand-washing facilities in the production area can potentially create a hygienic problem. This appears to be particularly important in the transmission of enteric viruses, such as Hepatitis A virus. NACMCF (1999) concluded that persons who harvest and/or process fresh produce should be viewed as food handlers rather than agricultural workers. Beuchat (1995) reported of outbreaks of *Shigella flexneri* and Hepatitis A. which could be traced back, to infected people working on the fields or in the packaging

facility. Clean, well-designed, and maintained equipment is less likely to cause damage to fresh produce and to introduce spoilage and pathogenic microorganisms (Brackett, 1992). On the contrary, dirty storage facilities and the presence of rodents, birds, and insects may increase the risk of contamination with foodborne pathogens (FDA, 1998). Finally, harvesting at the appropriate time and keeping the harvested product under controlled environmental conditions will help retard growth of post-harvest spoilage (Brackett, 1992) and pathogenic microorganisms.

2.9 Post harvest handling

Post harvest treatment of vegetables includes handling, storage, transportation and cleaning. During these practices, conditions may arise which lead to cross contamination of the produce from other agricultural materials or from the handlers. Environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption. Studies by Amoah *et al*. (2005, 2006) also indicate that there is increase evidence of possible post-harvest contamination especially in markets. Poor handling during harvesting, packaging and transportation can damage fresh produce, and enhance the susceptibility to the growth and survival of spoilage and pathogenic microorganisms. The presence of cut and damaged surfaces provides an opportunity for contamination and growth of microorganisms and ingress into plant tissues (Francis *et al*., 1999).

2.10 Health risks associated with wastewater use

A number of risk factors have been identified for the use of wastewaters in for agricultural irrigation. Some risk factors have short-term impacts and vary in severity depending on the potential for human, animal or environmental contact (e.g. microbial pathogens), while others have longer term impacts which increase with continued use of wastewater. The use of wastewater in vegetable farming facilitates the transmission of excreta-related diseases affecting human health. Based on epidemiological evidence, two major human health-related wastewater irrigation risks have been established: transmission of intestinal nematode infections such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and *Necator americanus* and transmission of faecal bacterial infections like *Escherichia coli (E. coli) diarrhoea*, *Typhoid*, *Salmonellosis* to farmers, produce consumers, and those living close to wastewater irrigated fields (Shuval *et al*., 1986; WHO, 1989). Studies in Mexico City and West Africa have shown high significance of *Ascaris* infections and diarrhoeal diseases in farm workers and enteric infections for consumers (Redwood, 2004). Transmission of diseases occurs from direct contact with farm workers and others in the fields especially children and from consumption of irrigated crops, especially vegetables eaten raw such as lettuce (Blumenthal and Goldberg, 2002).

2.11 Non-treatment interventions to protect consumer health

Health risks are a result of human exposure to bacteria, viruses, protozoa and helminths and also to toxic chemicals such as heavy metals. To abate the recurrence of health risks associated with wastewater irrigation, there is the need to treat wastewater before it is

used for irrigation purposes. To this end, World Health Organization (WHO, 1975) recommended that crops to be eaten raw should be irrigated only with biologically treated effluent which has been disinfected to achieve a coliform level of not more than 1000 coliform per 100 ml in 80% of the samples. This effective wastewater treatment can reduce pathogen levels. But in most developing countries it is not an option for the municipal authorities due to the high costs involved (Keraita *et al*., 2002).

Against this background, the WHO (2006) proposed the use of comprehensive risk assessment and management strategies that encompasses all steps in the process, from generation and use of wastewater to produce consumption. This can be done by constructing multiple barriers along the process pathway through the use of various risk management strategies which can have a cumulative effect. The integrated management of risks can include crop restrictions, application techniques that reduce produce contamination, the prevention of cross contamination, the promotion of improved food hygiene and handling, wastewater application techniques, human exposure control etc.

2.11.1 Wastewater application techniques

One of the factors influencing the microbial quality of farm produce, and thus health risks, is the mode of irrigation (Brackett, 1999). Based on health impacts from wastewater, WHO classified irrigation into three distinct categories: flood and furrow, spray and sprinkler and localised irrigation methods (WHO, 2006)

Flood and furrow irrigation methods apply water on the surface and pose the highest risks to field workers, especially when protective clothing is not used (Blumenthal and Peasey 2000b). Spray and sprinkler are overhead irrigation methods and have the highest

potential to transfer pathogens to crop surfaces, as water is applied to edible parts of most crops and also because aerosol-borne pathogens are carried further. According to Keraita *et al.* (2007b), overhead irrigation with watering cans with or without perforations at the outlet increases contamination on lettuce. Localised techniques, such as drip-and-trickle irrigation, present the lowest risk to farmers because water is directly applied to the root (Pescod, 1992). Localised irrigation is most expensive and prone to clogging of irrigation channels because of the turbidity of polluted water (Martijn and Redwood, 2005).

2.11.2 Cessation of irrigation

The interval between final irrigation and consumption could reduce pathogens by approximately 1 log unit per day (Petterson and Ashbolt, 2003). The precise value depends on climatic conditions, with more rapid pathogen die-off (approximately 2 log units per day) in hot, dry weather and less in cool or wet weather without much direct sunlight (approximately 0.5 log unit per day). Helminth eggs can remain viable on crop surfaces for up to two months, although few survive beyond approximately 30 days (Strauss, 1996).

Vas da Costa *et al.* (1996) showed that cessation of irrigation with wastewater for one or two weeks prior to harvest can be effective in reducing crop contamination by providing enough time for pathogen die-off. Enforcing withholding periods is likely to be difficult, however, in unregulated circumstances, because many vegetables (especially lettuce and other leafy vegetables) need watering until harvest to maintain their freshness and increase their market value. However, this may be possible with some fodder crops that do not have to be harvested at the peak of their freshness (Blumenthal and Peasey,

2000b). Alternatively, crops could be irrigated from non-contaminated water sources (where available) after until harvest.

2.11.3 Food preparation measures

2.11.3.1 Washing

Washing can lead to spread of bacteria. For example if only one leaf of a head of lettuce is contaminated, the washing process can transfer the bacteria to all the other leaves. However, vigorous washing of rough-surface salad crops (e.g. lettuce, parsley) and vegetables eaten uncooked in tap water reduces bacteria by at least one log unit. For smooth surfaced salad crops (e.g. cucumbers, tomatoes), the reduction is approximately two log units (Brackett, 1987; Beuchat, 1998; Lang *et al*. 2004). Washing lettuce, irrespective of the methods used for a least two minutes contact time reduces bacterial contamination (Amoah *et al*., 2007). Therefore, effective hygiene education and promotion programmes will be required to inform local food handlers (in markets, in the home and in restaurants and food kiosks) how and why they should wash wastewater irrigated produce effectively with water or disinfectant and/or detergent solutions.

2.11.3.2 Use of disinfectants

Various disinfectants can be used to reduce the microbial load on fruits and vegetables. However, the safety assessments of these substances and the legal requirements concerning such treatments also have to be taken into account. The purpose of using these agents is to control plant pathogens (plant protection), food pathogens, or spoilage organisms (preserving additive). The effect of disinfectants on contaminants depends on

many factors including the concentration used, treatment time, temperature, pH and sensitivity of the target organism(s). Chlorine is the major compound used for disinfection of fresh produce. During sprouting of seeds, chlorine can be used in the water to prevent growth of contaminating microorganisms. The most effective form is hypochlorous acid (HOCl) (Simons and Sanguansri, 1997) and the chlorine concentration of 100 ppm is frequently used. Washing in a disinfectant solution (commonly a hypochlorite solution) and rinsing in tap water can reduce pathogens by 1-2 log units. However, the use of chlorine does not ensure elimination or even an efficient reduction in pathogen levels. Indeed, already the removal of outer lettuce leaves reduces the faecal coliforms contamination level by 0.5–0.9 log units. Other substances many be used including organic acids, chlorine dioxide, hydrogen peroxide and ozone (Beuchat, 1998). Organic acids alone, or in combination with chlorine, have been shown in experimental designs to effectively reduce the number of pathogens for example, *Yersinia enterocolitica* and *Listeria monocytogenes* in parsley (Zhang and Farber, 1996). Beuchat (1998) concluded that prevention of contamination at all points of the food chain is preferred.

2.12 WHO guidelines or standard

Although irrigation with wastewater has been practised for centuries, the first regulations were developed in the early 20th century. With the growing awareness and fear of transmission of communicable diseases, strict guidelines were set. Following the recommendations by a WHO Scientific Group, WHO published guidelines for wastewater use in agriculture and aquaculture (WHO, 1989). The purpose of the

guidelines was to guide design engineers and planners in the choice of waste treatment technologies and waste management options.

The World Health Organisation's guidelines for microbiological quality for wastewater use in agriculture are based on "epidemiological evidence of actual risks to public health, rather than on potential hazards indicated by the survival of pathogens on crops and in the soil," (Mara and Cairncross, 1989). They were formulated for use in the design of wastewater treatment plants, but have subsequently influenced the standards for wastewater reuse for agriculture adopted in many countries.

Westcot (1997) addressed the question of how the WHO guidelines can be applied when there is little or no treatment of urban wastewater before it is used to irrigate crops. He suggests that, in the absence of better information, it is prudent to use the WHO standards for faecal coliforms as the quality standard. The WHO standards for intestinal nematodes and faecal coliforms in irrigation water are ≤1 egg/l and ≤1000 faecal coliform/100 ml (Hespanhol and Prost, 1994). The successful implementation of these guidelines depends on number of technical, economic, socio-cultural and institutional assumptions, which are mostly unachievable in poor countries like Ghana; hence the need for fine-tuning of the guidelines to meet local realities.

In many low-income countries, like Ghana, wastewater treatment as expected by the guidelines is not possible due to a variety of (mostly economics) reasons. The enforcement of the guidelines in such situations would stop hundreds or thousands of farmers irrigating along increasingly polluted streams and put their livelihoods at risk. Restrictions would also affect food traders and the general market supply with perishable crops, especially in cases where other water sources are (seasonally) unavailable.

It is similarly difficult to apply recommended additional health protection measures in market-oriented vegetable production system. In situations where highly specialized farmers cultivate cash crops according to market demand, crop restrictions would immediately threaten farmers' livelihoods.

2.12.1 The new WHO guidelines (WHO 2006)

Table 3 shows pathogen reductions achieved by several options for combining wastewater treatment and other health protection measures. This integrated risk assessment and management approach promoted by the WHO (2006) befits the situation in Ghana because it entails a comprehensive approach covering all steps in the process. It provides a basis for constructing multiple barriers along the production-consumption chain by using various non-treatment options to ensure health risk reduction associated with wastewater use. To reduce risk from pathogens, the new WHO guidelines focus on health-based targets which offer planners various combinations of locally possible risk management options for meeting them. These options go beyond those suggested in the previous guidelines and have to be used in combination as their impact, for example, on pathogen die off vary. Developing local guidelines requires comprehensive health protection barriers along the production-consumption pathway.

Table 3 Effectiveness of selected health- protection measures that can be used to remove pathogens (thermotolerant coliforms) from wastewater irrigated crops (WHO, 2006, modified)

Protection measure(examples)	Pathogen reduction (log units)
Wastewater treatment (to different degrees)	1-6
Localized (drip) irrigation(with 'low growing' crops, such as lettuce)	2
Localized (drip) irrigation (with 'high –growing' crops, such as tomatoes)	4
Pathogen die-off on the surface of crops after the last irrigation	0.5-2 per day
Washing of produce with clean water	1
Disinfection of produce (using a weak disinfectant solution)	2
Disinfection of produce (using one part vinegar and two parts water)	2
Peeling of produce (fruits, root crops)	2
Cooking of produce	6-7

Sources: Beuchat (1998); Petterson and Ashbolt (2003)

However, existing microbial studies on wastewater reuse in Ghana by Amoah *et al.* (2005, 2006) and Keraita *et al. (*2007a) have focussed on developing non-treatment options at farm and kitchen to reduce vegetable contamination. All these studies used lettuce and cabbage as the test crop whilst spring onion which is one of the vegetable eaten raw has not been considered. There is presently no detailed work done at market to test different non-treatment options in reducing microbial contamination on spring onions neither do farm and kitchen. Thus, a holistic view of reducing microbial contamination on wastewater irrigated spring onions in the production-consumption pathway will give a

more systematic approach in assessing the effectiveness of the proposed standards developed by WHO (2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Organization of the study

The research was carried out in three stages. The first stage addressed the effectiveness of cessation of irrigation on spring onion on farm as well as the effect of watering heights on contamination levels. The portion of spring onion more prone to contamination was also ascertained. The second stage assessed different post harvest handling practices to identify appropriate and feasible interventions that can reduce thermotolerant coliforms and helminth egg numbers on spring onions. The third stage assessed the effectiveness of combining feasible interventions selected from the farm to kitchen pathway.

3.2 Study area

Kumasi is the capital town of Ashanti Region and the second largest city in Ghana. It has a population of about one million people with an annual growth rate of 5.9% (Ghana Statistical Service, 2002) and is one of Africa's growing urban centres. It lies between latitude 6° 42" North and longitude1°35" West, and approximately 260 m above sea level. The city covers a total area of 57 km^2 and has terrains that vary from gently undulating to distinctly hilly and mountainous (Taylor *et al.*, 2000).

The city has a semi-humid tropical climate and lies in the tropical forest zone. There are two major seasons in the city, the rainy and dry seasons. The rainy season experiences major rains between March and July and minor rains between September and November with an annual rainfall of about 1300 mm. The main dry season occurs

between the month of November and March with total average rainfall of 160 mm. The relative humidity ranges between 75-79% with average daily sunshine durations ranging between 2 to 7 hours and daily minimum and maximum temperatures of 21.20°C and 35.50°C, respectively (Meteorological Services Department, 2002).

In urban Kumasi, most land where farming is done belongs to government institutions, private developers etc. There are about 41 hectares in the urban area under vegetable irrigation while the peri-urban area has more than 12,000 hectares under irrigated vegetable farming mostly during the dry season (Cornish *et al.*, 2001). Agriculture remains an important livelihood component for many peri-urban residents. Peri urban agriculture is becoming typically more intensive as the urban area grows in size and agricultural production emphasis shifts towards high value, perishable products such as vegetables, which come with a ready urban market. Urban vegetable farmers in search for irrigation water usually have no alternative than to use polluted water, readily available for irrigation due to scarcity of fresh water.

The study was conducted in the dry season at selected production sites ('Quarters' farm and 'D-line' farm), vegetable selling points (Racecourse, European and Ayigya market) and street food vendor sites (KNUST, Oforikrom, and Kentinkrono) in Kumasi.

3.3 Vegetable of study

Spring onion was chosen for the study. The type of spring onion grown in Ghana is normally imported from Burkina Faso which farmers claim gives good yield. Some farmers in Ghana cultivate spring onions by splitting the cluster of already matured spring onion and plant into single strands while others sow the seeds directly on the field.

Those cultivated with matured spring onions take 6 to 7 weeks to mature while those sown with seeds take 10-12 weeks. The advantage of using seeds is that it gives better yield compared to those planted with matured spring onion.

3.4 Production sites

Two sites were chosen for the study. The 'Quarters' farm which is located at Gyinyase is the largest urban vegetable-farming site in Kumasi (21.8 ha) (Figure 1). It is situated next to the Kwame Nkrumah University of Science and Technology (KNUST) in an inland valley. It lies between latitude 06˚39'44"N and longitude 01˚34'38"W. About 60 vegetable farmers grow a diversity of crops and practice some form of organic farming. There is a well-established farmer's organization (Obuobie *et al.*, 2006). The main source of water for irrigation is hand dug shallow wells, which are less than 1 m deep and watering is done with watering cans. Some of the crops grown on this farm include lettuce, spring onions, cabbage, green pepper, and carrots. The second site 'D-line' is located behind the local university (KNUST) police station. This site is located at 06˚41'14"N and longitude 01˚33'58"W. There are about 20 farmers with a total cultivation area of about 3 ha (Figure 3.2). Farmers predominantly cultivate spring onions. The main source of water for irrigation is a stream, which has been impounded at different points to enhance easy fetching of water by the farmers.

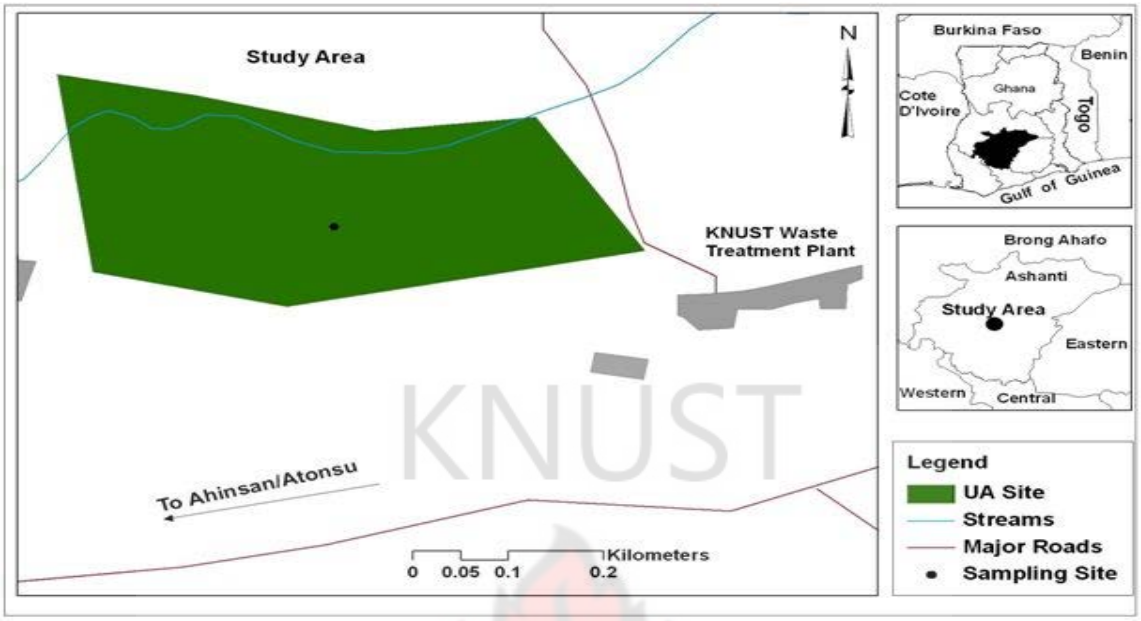

Figure 2: Water and spring onion sampling site at 'Quarters' farm (after Amoah, 2008)

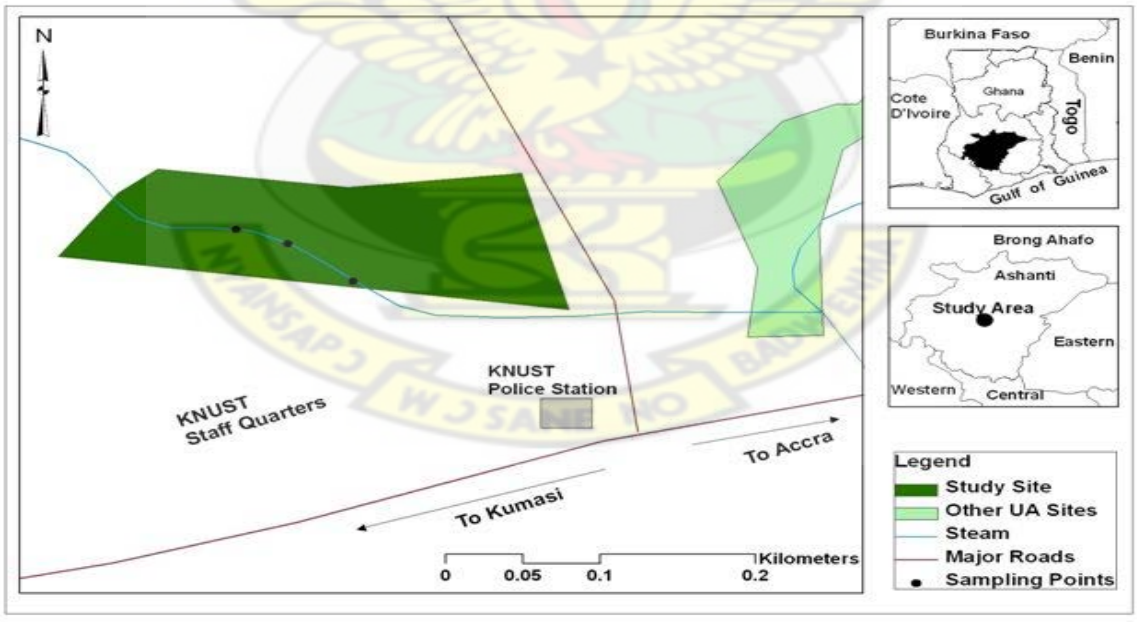

Figure 3: Water and spring onion sampling sites at 'D-line' farm (after Amoah, 2008)

3.4.1 Cessation of irrigation at production sites

Cessation of irrigation as a component of multiple barrier approach has not been tried in Ghana on spring onions as it is in the case of lettuce. At the production sites, cessation of irrigation was tried on spring onions as a pre-test to establish the number of days irrigation could be withheld without necessarily affecting produce quality.

3.4.2 Experimental design

On the two farm sites, the same experiment was carried out in collaboration with the farmers. Treatments were arranged in Randomized Completely Block Design (RCBD).

Table 4: Field layout of the four non-treatment intervention options tested

Bed 1	Bed 2	Bed 3	Bed 4
D0	D2	D4	D6
D2	D4	D2	D0
D4	D6	D0	D2
D6	D0	D6	D4

Irrigate until harvesting (D0), Irrigation stoppage two days prior to harvesting (D2), Irrigation stoppage four days prior to harvesting (D4), and Irrigation stoppage six days prior to harvesting (D6).

The trial comprised of four beds with each bed divided into four sub plots. Treatments were designed at 2-day intervals for up to 6 days cessation of irrigation prior to harvesting at 'Quarters' farm. These treatments were allocated to each sub plot as follows: irrigating until harvesting (D0), stop irrigating two days prior to harvesting (D2), stop irrigating four days prior to harvesting (D4), and stop irrigating six days prior to harvesting (D6). Each treatment was randomised in other sub plots of the other beds for all the four beds constituting one block. This was replicated at 'D-line' farm. Each sub plot covered an estimated area of 10 m^2 with about 100 heads of spring onion plants. The

same water source was used to irrigate all the treatments at each trial. The farmers did all work as part of their daily schedule. Farming practices did not vary much between farms. Sampling was done between November and April 2008. Spring onions are generally tied up in bunches. A bunch as used in this study refers to ten heads of spring onion plant. In the laboratory, the roots of the spring onions were trimmed off with a sterile stainless steel knife. The bulbs and leaves were shredded and well mixed to obtain a homogeneous (composite) sample for all analyses carried out in the laboratory unless otherwise stated.

3.4.3 Sampling of spring onions from production sites

Three bunches of spring onions were randomly sampled at the middle potion of each sub plot to prevent border effect. Samples were taken from 'Quarters' farm and placed in separate sterilized polythene bags. This was transported in ice box to the laboratory. Triplicate sub samples from each treatment were taken for thermotolerant and helminth eggs analysis in the laboratory. The same sampling procedure was replicated at 'D-line' farm. Irrigation water samples were taken into sterilized bottles from both production sites and conveyed on ice to the laboratory and analyses carried out within one hour.

3.4.4 Effect of irrigation stoppage on yield loss of spring onions

Fresh weights were taken by weighing five bunches of spring onion from each sub plot at 'Quarters' farm and an average weight was recorded. The same sampling procedure was replicated at 'D-line' farm. Based on the laboratory results and the outcome of Focus Group discussions with the farmers about their perception on cessation of irrigation, a

two-day (D2) irrigation stoppage period was selected as the best (adoptable) option for spring onion as it seemed to have the greatest adoption potential.

3.5 Sources of contamination on farm

3.5.1 Distribution of thermotolerant coliforms and helminth eggs on spring onion parts

To determine the major source of contamination, two bunches of spring onions were sampled at 'Quarters' farm and conveyed on ice to the laboratory. Spring onion samples were shredded into leaves and bulbs separately. Triplicate sub samples were taken from these parts and analysed for thermotolerant coliforms and helminth eggs. The same sampling procedure was replicated at 'D-line' farm.

3.6 Managing watering can-based irrigation to reduce contamination

The watering can method is the common irrigation method used by farmers at the two production sites. Farmers usually lift watering cans at different heights convenient to them during irrigation. As a way of reducing contamination, it was proposed that spring onions be irrigated at the base i.e. at less than 0.5 m high. The control experiment was to irrigate spring onions at a height of about 0.75 m and greater than 1.0 m within which farmers can lift the watering can.

At each of these heights, watering cans with and without perforated caps were used to water already matured spring onion plants. On each sampling day, spring onion samples from each treatment (<0.5 m, 0.75 m, and >1.0 m)) for both watering cans with or without perforations were taken immediately after watering and transported to the laboratory in an icebox containing icepacks, for thermotolerant coliforms and helminth

eggs analysis. Triplicate sub samples were taken and were replicated three times given 12 samples for each treatment.

3.7 Market study

3.7.1 Post-harvest handling practices and assessment of interventions of spring onions in the markets study sites.

At the market site, an initial feasibility study was carried out to understand the current handling of spring onions so as to develop appropriate interventions. The objective of the study was to document handling practices of spring onion and use as the basis for developing feasible and practical non-treatment interventions that will significantly reduce consumer related health risk from wastewater-irrigated spring onions.

The observational study was made in two major markets (Racecourse and European) and one neighbourhood market (Ayigya) within the Kumasi metropolis. The market selection criteria included: market structure, environment, size, and number of irrigated vegetable sellers, handling practices, types of vegetables sold, availability of potable water, etc. Based on these criteria the markets were classified into low (e.g. Racecourse), middle (Ayigya) and high class (European) markets. In-depth observation and data collection was done at racecourse market since is the main depot for vegetables within the Kumasi metropolis.

3.7.2. Racecourse market

This is one of the biggest markets in the city hosting thousands of people from all corners of the city. It is an unplanned open market with ground untarred which makes it muddy

during the rainy season and very dusty during the dry season. There are no properly constructed drains therefore wastewater generated by the market women and neighbouring households is directly released into the market area. There are heaps of rubbish piled indiscriminately around the market area. The whole market has two public toilets, which are not properly managed, and so gives off very bad stench even at a distance further away (20 m) from the place of convenience. There are about 50 vegetable sellers grouped at one place. The types of vegetable sold include lettuce, spring onions, cabbage, green pepper, etc. There is a stand pipe about 10 m away from the vegetable selling point and other boreholes are located further away. Sellers normally go to the farm to harvest spring onions in the afternoon after their daily sales. The harvested spring onions are stored and tied in sacks/fertilizer bags. The packed spring onions are kept along the roadside or the seller's house until the next morning (dawn) when it is conveyed to the market by means of taxi or mini bus transport system. At the market, wholesalers keep heaps of spring onions under tables from which they pick to display on tables for sale. Retailers on the other hand, display spring onions in bowls and in baskets for sale.

3.7.3. European market

The market is located in the central part of the city. It is a tarred area with no heaps of refuse piled around. It is a well-organized market with each vegetable seller having a stall where vegetables are kept. About 10 irrigated vegetable sellers deal in different types of vegetables like lettuce, cabbage, spring onions, carrots, cauliflower, cantaloupe etc. Almost all vegetables are washed before display for sale. For spring onions, sellers wash

the bulbs where pockets of soil particles are adhered to in a bowl of water and display them vertically in baskets for sale. There is a stand pipe located about 20 m from the vegetable selling point.

3.7.4 Ayigya market

It is a neighbourhood market with about 20 vegetable sellers scattered all over. It is a well-constructed market with tarred pavements even though some portions are rough with pot holes. There is a public toilet and stand pipe about 10 m away from the market. Sellers displayed their vegetables in front of their mini shops. No form of washing is given to spring onions in this market.

3.8 Street food vendor sites

At the kitchen, vegetable handlers/food vendors normally treat spring onions with salt or vinegar solution. Concentration of sanitizers used is exclusively on the discretion of vegetable handlers. For the purpose of this study, a concentration of 7 ppm salt solution and 100 ml of vinegar (manufactured by P and M farms Limited): 500 ml of water was used for sanitizing spring onions at the kitchen. These concentrations were used based on the recommendations made by Amoah *et al.* (2007) in effectiveness of common and improved sanitary washing methods for the reduction of coliform bacteria and helminth eggs on vegetables.

3.9 Evaluation of non-treatment interventions

Based on the observations made at the markets and kitchen, the following non-treatment interventions were assessed for their effectiveness in reducing thermotolerant coliforms and helminth egg numbers.

3.9.1 Post-harvest related non-treatment interventions (market)

3.9.1.1 Effect of storage materials on spring onions

Spring onions are generally harvested the previous day, tied and stored in sacks overnight. The proposed intervention was to store spring onions in baskets overnight instead of the sacks. Samples were taken from spring onions stored in fertilizer bag and those stored in basket overnight. Freshly harvested spring onions were also sampled from the field that morning to serve as control. All samples were taken randomly taken from the same bed. The samples were transported to the laboratory in cold icebox and triplicate sub samples were taken from the different storage materials for thermotolerant and helminth eggs analysis. This was replicated three times given 12 samples for each treatment.

3.9.1.2 Assessment of contamination loads in relation to modes of display of spring onions in the market.

Spring onions tied up in sacks are mostly untied and kept under tables before they are displayed for sale at the market. Sellers pick from this stock of spring onions and display on tables, bowls, and baskets. Samples were taken from these batches of spring onions after display for two to three hours, which is a typical turnover point in the market.

Samples were transported to the laboratory and triplicate sub samples were taken from each of the treatment for thermotolerant and helminth eggs analysis. This was replicated three times to assess the effect of placement on contamination loads. No new intervention was proposed for placement of spring onions since discussions with sellers did not generate other intervention options.

3.9.1.3 Effectiveness of washing spring onions in the market

Spring onions are generally not washed or refreshed in the markets before placement unlike in the case of lettuce. The proposed interventions were washing one (1 kg) of whole spring onion plant in half bucket of water (8.5 litres) and washing under running tap water for two minutes. Additionally, one (1 kg) of spring onion bulb was washed in half bucket of water (8.5 litres) for the same period of time. Triplicate sub samples were taken and also from the unwashed spring onion to serve as control. This was replicated three times given 12 samples for each treatment. These samples taken from the same original stock were analysed in the laboratory for thermotolerant coliforms and helminth eggs and the results compared.

3.9.1.4 Effect of continuous washing of different stock of spring onions (bulbs) in the same amount of water in the market

Washing of spring onion bulbs was carried out to determine how many times different batches of the bulbs could be washed in the same amount of water to effectively reduce thermotolerant and helminth eggs numbers. For each treatment, spring onion bulbs weighing one (1 kg) were washed in half bucket (8.5 litres) of water for two minutes.

Subsequently, another portion of 1 kg of spring onion bulb was washed in the same half bucket of water for five consecutive times. Samples were transported to the laboratory on ice for thermotolerant coliforms and helminth eggs analysis. Triplicate sub samples were then taken from these batches to assess the effect of continuous washing on contamination levels. These were pooled from the same original stock with two replications for each treatment.

3.9.1.5 Handling practices in the various markets and implications

Although spring onions did not come from the same farm, handling practices varied at the three different markets. For example, European market sellers washes the bulbs of spring onions, which may have pockets of soil particles attached while Ayigya and Racecourse did not do any form of washing. Three samples were taken from the three markets and samples were stored in an icebox containing icepacks and processed in the laboratory for thermotolerant coliforms and helminth eggs. Triplicate sub samples were taken from these spring onion samples to compare the effect of handling practice. This was replicated three times given a sample size of 12 for each treatment. Handling practice at European market was however, used as the best and feasible intervention for reducing thermotolerant coliforms and helminth egg numbers on spring onion at the market.

3.10 Post harvest related non-treatment interventions (kitchen)

3.10.1 Efficacy of sanitizers on spring onion decontamination

Three sellers each were randomly selected from KNUST, Oforikrom and Kentinkrono. Samples were taken from spring onions before and after use of sanitizers (salt and

vinegar) and transported on ice to the laboratory for thermotolerant and helminth eggs analysis. Triplicate sub samples were taken from these batches to assess the efficacy of sanitizers on spring onions. This was replicated two times given 9 samples for each treatment.

3.11 Selected feasible interventions from production sites to the kitchen

After laboratory analysis and informal Focus Group discussions with the various stakeholders, the following interventions were proposed from production site to street food vendor site: For the production site, a 2-day (D2) cessation of irrigation of spring onion was selected since reducing watering heights to minimize contamination could not yield the expected results for spring onion. This was followed by storage of spring onions in basket overnight along the road side to be transported to the market. At market, bulbs of spring onion was washed in a bowl of water (8.5 litres) for two minutes and displayed vertically in basket as is normally practiced at the European market. At the kitchen, 100 ml of vinegar: 500 ml of water and salt solution with concentration of 7 ppm were used as the main sanitizers.

3.11.1 Tracking contamination loads along the production-consumption pathway of wastewater irrigated spring onions.

Spring onion samples were followed from farm to the market, and to the kitchen and the selected interventions applied along the pathway. On each sampling date at 'Quarters' farm, ten bunches of spring onions, which had been exposed to 2-day (D2) cessation of irrigation on farm were harvested in the afternoon and stored overnight in baskets. The

market woman who bought the stock of spring onions was then followed to the Racecourse market. At Racecourse market, 1 kg of the samples were taken from the original stock and exposed to the market interventions as follows: the bulbs of spring onions (1 kg) were washed in half bowl of water (8.5 litres) for two minutes, and displayed in basket for at least 2-3 hours. The food vendor who bought the washed spring onion was followed to the kitchen. In this kitchen, the food vendor washed the spring onions in 100 ml vinegar: 500 ml of water for 10 minutes. Another stock from the same bunch of spring onions was washed in salt solution (7 ppm) concentrations for 10 minutes. All samples were stored in an icebox containing icepacks and processed in the laboratory for thermotolerant coliforms and helminth eggs. Triplicate sub samples were taken from these batches of spring onions before and after they have been subjected to the various non-treatment interventions from the farm, market and the kitchen.

3.12 Thermotolerant coliforms and helminth egg counts

3.12.1 Enumeration of Thermotolerant coliforms in irrigation water and spring onion

Thermotolerant coliform counts were estimated using a three-tube Most Probable Number method (MPN) according to standard procedures (Anon, 1998). About 10 g (fresh weight) of spring onion was weighed into a stomacher bag and pulsified in 90 ml of 0.9 % NaCl solution for 30 seconds using a pulsifier (model number PUL 100E and manufactured by Microgen Bioproducts Limited in the United Kingdom with serial number 230 03 071). Serial dilutions of $1:10^{10}$ were made and triplicate tubes of MacConkey broth supplied by MERCK (Germany) were inoculated with the desired

aliquots from each dilution prepared. The mixture (e.g. 1 millilitre aliquots from the stomacher bag, in 5 ml of MacConkey broth) containing an inverted Durham tubes was incubated at 44 °C for 18-24 hr. Tubes showing colour change from purple to yellow and gas collected in the Durham tube after 24 hr were identified as positive. From each of the positive tubes identified, a drop was transferred into a 5 ml test tube of trypton water and incubated at 44 ^{o}C for 24 hr after a drop of Kovacs' reagent was added to the tube of trypton water. All tubes showing a red ring colour development after gentle agitation denoted the presence of indole and were recorded as presumptive positive for thermotolerant coliforms. The tubes that maintained the yellow colour after the addition of Kovacs' reagent were recorded negative for thermotolerant coliforms. Counts per 100 ml were calculated from Most Probable Number Tables (Anon, 1998)

3.12.2 Enumeration of helminth eggs in irrigation water and on spring onions

Helminth egg population in irrigation water and spring onions were determined using the flotation sedimentation method, which is a US-EPA method by Schwartzbrod (1998).

The reagents used were prepared as follows: 1) 573 g of zinc sulphate (Harris reagent; Philip Harris plc, Shenstone, England) was dissolved completely in one litre of sterilized deionised water to produce zinc sulphate solution of specific gravity of about 1.2, and (2) acid/alcohol buffer solution was prepared by adding 5.16 ml H_2SO_4 to 350 ml of ethanol. Sufficient deionised water was then added to the acid/alcohol mixture to produce 1 litre of the solution.

3.12.4 Helminth eggs in irrigation water

Irrigation water samples were allowed to settle overnight or at least for three hours. This was to enable the helminth eggs settle under their own weight. Much of the supernatant as possible was sucked up and the sediment transferred into 15 ml centrifuge tubes. The 2-litre containers were rinsed 2-3 times with deionized water and the rinses were transferred into centrifuge tubes. The tubes were then centrifuged at 1,450 rpm for three minutes. The sediments in the centrifuge tubes for each sample were pooled into one centrifuge tube and centrifuged again at 1,450 rpm for three minutes.

The supernatant was poured away and the deposit was re-suspended in about 150 ml $ZnSO_4$ (372 g/l, density of 1.3). The mixture was homogenized with a spatula and centrifuged at 1,450 rpm. At a density of 1.3 ($ZnSO_4$), all helminth eggs float leaving other sediments at the bottom of the centrifuge tube. The $ZnSO_4$ supernatant (containing the eggs) was poured into a 2-litre flask and diluted with at least one litre of water. This was allowed to settle overnight for the eggs to settle again. As much supernatant as possible was sucked up and the deposit was re-suspended by shaking. The resuspended deposit was put into centrifuge tubes. The 2-litre container was rinsed 2-3 times with deionised water and the rinsed water added to the centrifuged tubes and centrifuged at 1600 rpm for three minutes. The deposit was pooled into one tube and centrifuged again at the same speed and for the same period of time.

Thereafter, the deposit was re-suspended in acid/alcohol (H_2SO_4 + C_2H_5OH), after sucking much of the supernatant, and concentrated ethyl ether was added. The mixture was shaken and the centrifuge tube occasionally opened to let out gas before centrifuged at 2200 rpm for three minutes. After the centrifugation, a diphasic (lipophilic

and aqueous phase representing the ethyl ether and acid/alcohol, respectively) solution was formed. With a micropipette, as much of the supernatant as possible (starting from the lipophilic and then the aqueous phase) was sucked up leaving about 1 ml of deposit. The deposit was observed on a Sedgwick-Rafter cell under the microscope (x100) and the eggs counted.

3.12.5 Helminth eggs on spring onion

About 100 g (fresh weight) of spring onion was washed in about 1 litre of water. The spring onions were rinsed with water and the washed solution made up to at least 2 litres. The washed water was analyzed for helminth egg as described above.

3.12.6 Calculation

The number of eggs per litre was calculated from the equation:

$N = (AX)/ (PV)$

Where N = Number of eggs per litre of sample

A = number of eggs counted in the slide or mean counts from two or three slides

X = volume of the final product (mL)

P = volume of the slide (mL)

V = original sample volume (L)

3.12.7 Identification of helminth eggs

The helminth eggs were identified based on their shape and size and compared with standard eggs on a chart prepared by WHO (1996).

3.13 Statistical analysis

The Statistical Package for Social Scientists (SPSS) version 13.0 was used for testing the various statistical relationships between and within treatment variables. Thermotolerant coliform numbers (MPN) were transformed into logs, prior to subjecting the data to analysis of variance. T-tests were used where appropriate. Results of analysis were quoted at $P \leq 0.05$ level of significance.

CHAPTER FOUR

RESULTS

4.1 Irrigation water quality at production sites

Table 5 shows that irrigation water sampled from 'Quarters' farm (shallow hand dug well) and 'D-line' farm (stream) contained Geomean of thermotolerant coliforms of 4.21 and 3.12 $\log_{10}$ units /100ml and helminth eggs of 1.1 and 0.3 eggs l^{-1}, respectively. Statistically, there were significant differences in thermotolerant coliforms ($\rho = 0.004$) and helminth egg numbers ($\rho = 0.013$) at both production sites (Appendix A1 and A2).

Table 5: Thermotolerant coliforms and helminth egg numbers in irrigation water at the two production sites

Production sites	Thermotolerant Coliforms (Log_{10} MPN/100ml ± S.E) [a]	(No. of Helminth eggs/ 100 ml sample)[b]
'Quarters'	4.21 (± 0.22)[c]	1.1 (± 0.21)
'D- line'	3.12 (± 0.24)	0.3 (±0.13)

a Geometric mean (n =8 for each irrigation water source)
[b]Arithmetic mean
[c] Figures in parentheses represent the standard error

4.2 Effect of irrigation cessation time on thermotolerant coliforms, helminth egg numbers, and fresh weight of wastewater-irrigated spring onion

Table 6 shows irrigation cessation times in (D0-D6) days for thermotolerant coliforms, helminth eggs, and fresh weight of spring onions.

Table 6: Thermotolerant coliforms, helminth egg numbers and mean fresh weights of spring onions at both production sites

Cessation time (days)	Thermotolerant coliforms (Log_{10} MPN/100g ±S.E)[a]		(No. of Helminth eggs/ 100 g sample)[b]		Mean fresh weight (kg)[b]	
	Quarters	D-line	Quarters	D-line	Quarters	D-line
D0	5.84 (±0.28)[c]	4.92 (±0.24)	1.26 (±0.26)	1.1 (±0.15)	2.0 (±0.13)	1.98 (±0.09)
D2	4.74 (±0.11)	3.93 (±0.20)	1.06 (±0.15)	0.9 (±0.13)	1.67 (±0.12)	1.57 (±0.03)
D4	3.79 (±0.44)	3.03 (±0.14)	0.93 (±0.11)	0.8 (±0.15)	1.36 (±0.10)	1.46 (±0.01)
D6	2.34 (±0.52)	2.09 (±0.54)	0.88 (±0.15)	0.7 (±0.20)	1.05 (±0.01	1.11 (±0.05)

a Geometric mean (n=96 for cessation of irrigation, n=32 for fresh weights)
b Arithmetic mean
[c] Figures in parentheses represent the standard error

Generally, thermotolerant coliforms, helminth egg population, and fresh weights of spring onion decreased with an increase in the number of days after cessation of irrigation. Irrigation stoppage decreased thermotolerant coliforms at 'Quarters' farm significantly ($\rho = 0.000$) but not for helminth eggs ($\rho = 0.441$). Similarly, cessation of irrigation prior to harvesting at 'D-line' farm decreased thermotolerant coliforms significantly ($\rho = 0.000$) but there were no significant ($\rho = 0.474$) decrease in helminth egg numbers. An average daily reduction of 0.6 log unit for thermotolerant coliforms and 0.06 helminth eggs were obtained after D0-D6 cessation irrigation times. Significantly higher ($\rho = 0.000$) fresh weights of spring onion were recorded at both production sites at D0-day cessation of irrigation compared to that of D6-day cessation of irrigation

(Appendix A4 and A6). An average fresh weight of 0.15 kg was lost daily from cessation of irrigation before harvesting.

4.3 Management of watering can irrigation to reduce thermotolerant coliforms and helminth egg numbers on spring onion at production site

Table 7 shows that watering cans without perforated caps (uncapped) at the outlets recorded higher numbers of thermotolerant coliforms and helminth eggs on spring onions than those fitted with perforated caps (capped). Generally, using watering can to irrigate spring onions from different heights (< 0.5 m, 0.75 m, >1.0 m) with or without perforated caps showed significant variation in thermotolerant coliforms but not for helminth eggs. Thermotolerant coliform numbers for watering can with perforated caps at the outlet decreased significantly ($\rho = 0.001$) from that of < 0.5 to > 1.0 metres high. However, the decrease in thermotolerant coliform numbers between irrigation heights 0.75 and >1.0 metres were not significant ($\rho = 0.237$). In the same way, there was no significant decrease ($\rho = 0.151$) in helminth egg numbers between the different heights (< 0.5, 0.75, >1.0 m). Watering can without perforated caps at the outlet also recorded a significant decrease ($\rho = 0.000$) in thermotolerant coliforms between the irrigation heights (< 0.5, 0.75, >1.0 m). However, there was no significant ($\rho = 0.439$) difference in thermotolerant coliforms between irrigation heights 0.75 and >1.0 metres. In addition, there was no significant ($\rho = 0.750$) decrease in helminth egg numbers between the three different heights. Regardless of the watering heights and nature of outlets of watering can, no significant differences were obtained in terms of helminth egg numbers (Appendix A8 and A9).

Table 7: Numbers of thermotolerant coliforms and helminth eggs on spring onions irrigated at different heights with or without perforated caps

Irrigation	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E) [a]		(No. of Helminth eggs/ 100 g sample)	
Height (m)	Capped*	Uncapped**	Capped*	Uncapped**
<0.5	5.91 (±0.19) a	6.13 (0.19) a	1.1 (±0.19) a	1.3 (±0.18) a
0.75	5.25 (±0.15) b	5.35 (0.15) b	0.9 (±0.17) a	1.2 (±0.15) a
>1.0	4.99 (±0.14) b	5.17 (0.14) b	0.6 (±0.21) a	1.0 (±0.12) a

Figures in parentheses represent the standard error (n= 72)
Capped*-watering cans used in irrigation were fitted with perforated caps at the outlet.
Uncapped**- watering cans used had no perforated caps at the outlet.
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean
Numbers in the same column with the same letters showed no significant difference between the irrigation heights at ($\rho \leq 0.05$).

4.4 Distribution of thermotolerant coliforms and helminth eggs on spring onions

In Table 8 samples from leaves and bulbs of spring onions all contained 4.20 and 1.53 log_{10} units /100 ml of thermotolerant coliforms and helminth eggs of 3.5 and 1.0 eggs l^{-1} respectively. Bulbs of spring onion, (usually in contact with the soil) had significantly higher numbers ($\rho = 0.000$) of thermotolerant coliforms and helminth eggs (Appendix A7). The mean difference in numbers between the bulbs and leaves was 2.67 log units of thermotolerant coliforms and 2.5 helminth eggs.

Table 8: Thermotolerant coliforms and helminth egg numbers on bulbs and leaves of spring onion

Spring onion parts	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E) a	(No. of Helminth eggs/ 100 g sample) [b]
Bulbs	4.20 (±0.11) [c]	3.5 (±0.79)
Leaves	1.53 (±0.27)	1.0 (±0.19)

a Geometric mean (n=12)
b Arithmetic mean
[c] Figures in parentheses represent the standard error

4.5 Post-harvest handling practices and effectiveness of selected interventions at market sites

4.5.1 Storage material

Generally, basket as a storage material had significant effect on both thermotolerant coliforms (ρ=0.000) and helminth eggs contamination (ρ=0.000) (Table 4.5).

Table 9: Thermotolerant coliforms and helminth egg numbers on stored spring onions

Storage	Thermotolerant coliforms on (Log_{10} MPN/100g ± S.E) [a]	(No. of Helminth eggs/ 100 g sample)
Baskets	5.78 (±0.11) a	1.0 (± 0.13) a
Sacks	7.46 (± 0.21) b	1.9 (±0.22) a
Freshly harvested	7.57 (±0.19) b	2.1 (±0.20) a

Figures in parentheses represent the standard error (n=36)
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean
Mean values in the same column with the different letters are significant at $\rho \leq 0.05$

However, spring onions stored in sacks and those freshly harvested from the field did not show any significant difference ($\rho = 0.676$) in thermotolerant coliform and helminth egg ($\rho = 0.961$) numbers (Appendix B1i)

4.5.2 Displaying points

Table 10 shows thermotolerant coliforms and helminth egg numbers on spring onions after three-hour display in the market. Display of spring onions in baskets, bowls, tables, and no display (kept under tables) did not show any significant effect on thermotolerant coliform numbers ($\rho = 0.791$) and helminth egg ($\rho = 0.104$) numbers (Appendix B2).

Table 10: Thermotolerant coliforms and helminth egg numbers on displayed spring onions

Display points	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E) [a]	(No. of Helminth eggs/ 100 g sample)
Baskets	7.07 (±0.17)	1.4 (±0.2)
Bowls	7.19 (±0.20)	1.5 (±0.4)
Table	7.25 (±0.19)	1.6 (±0.3)
No display	7.52 (± 0.12)	2.2 (±0.2)

Figures in parentheses represent the standard errors (n=48)
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean

4.5.3 Washing of one (1) kg spring onions (whole plant and bulbs) at the market

Reduction in both thermotolerant coliforms and helminth egg numbers on whole plant of spring onions and bulbs after washing are shown in Table 11. Generally, thermotolerant coliform and helminth egg numbers reduced significantly ($\rho = 0.000$) after washing whole plant of spring onion under running tap than washing in a bowl of water (8.5 litres)

(Appendix B3). There was a significant difference (ρ = 0.001) in thermotolerant coliforms between washing whole plant of spring onions in bowls and under running tap. Washing of whole plant spring onion in a bowl and under running tap reduced thermotolerant coliform numbers by 3.89 and 4.75 log units, respectively. Additionally, helminth egg reductions achieved for washing spring onions in a bowl and under running tap was 1.4 and 2.8 eggs per 100 g wet weight respectively. Similarly, both thermotolerant coliform and helminth egg numbers reduced significantly (ρ = 0.000) when spring onion bulbs were washed under running tap than in a bowl of water (8.5 litres) (Appendix B4).

Table 11: Thermotolerant coliforms and helminth egg numbers on washed spring onion whole plant and bulbs for two minutes

Washing practices	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E) [a]		(No. of Helminth eggs/ 100 g sample)	
	Whole plant	Bulbs	Whole plant	Bulbs
Washing under running tap (2 min)	2.62 (±0.10)	1.69 (±0.09)	0.0 (±0.00)	0.0 (±0.00)
Washing in a bowl of water (8.5 litres for 2 min)	3.48 (±0.70)	2.50 (±0.16)	1.4(±0.15)	0.73 (±0.19)
Unwashed	7.37 (±0.22)	4.92 (±0.24)	2.8 (± 0.69)	3.13 (±0.26)

Figures in parentheses represent the standard error (n=72)
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean

4.5.4 Changes in thermotolerant coliforms and helminth egg numbers on spring onion bulbs washed repeatedly in the same amount (8.5 litres) of water.

Figure 4.1 shows effect of washing of bulbs of spring onion in 8.5 litres of water for two minutes in the same amount of water compared to no washing (unwashed).

Thermotolerant coliforms and helminth egg numbers increased as bulbs of spring onion were successively washed in one washing cycles without changing the water after each use. The efficacy of the decontamination process decreased after washing a total 5 kg spring onion bulb (in five cycles at 1 kg per cycle) in the same amount of water.

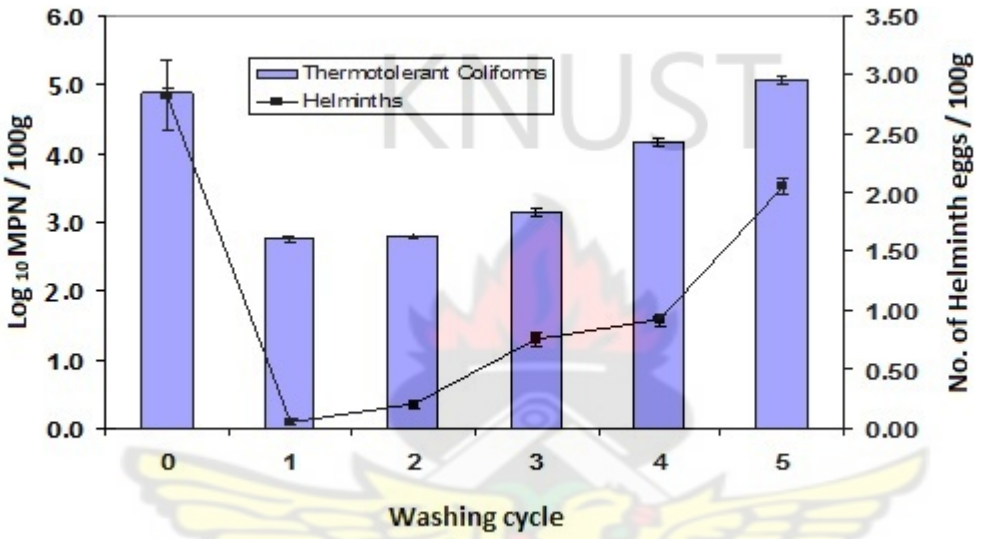

Figure 4.1: Effect of continuous washing of spring onion bulbs in the same amount of water (n=54)

(0) Unwashed spring onions (control); (1) First washing cycle; (2) Second washing cycle; (3) Third washing cycle; (4) Fourth washing cycle; and (5) Fifth washing cycle.

Thermotolerant coliform numbers on bulbs of spring onions increased significantly (ρ = 0.000) from the first to the fifth washing cycle. Although there was a decrease in thermotolerant coliform numbers from the first to the second cycle, the decrease was not significant (ρ = 0.855).

4.5.5 Role of the market environment

There were significant differences in both thermotolerant coliforms ($\rho = 0.000$) and helminth eggs ($\rho = 0.002$) on the spring onions sampled from the various markets (Table 12) (Appendix B6).

Table 12: Influence of market conditions on spring onion contamination

Market Environment	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E) [a]	(No. of Helminth eggs/ 100 g sample)
European	4.45 (± 0.14) a	0.6 (± 0.2) a
Ayigya	6.53 (±0.11) b	2.0 (±0.5) b
Racecourse	7.17 (±0.22) b	3.0 (±0.2) b

Figures in parenthesis represent the standard error (n=36)
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean
Mean values in the same column with the different letters are significant at $\rho \leq 0.05$

Significantly higher thermotolerant coliforms and helminth egg numbers were recorded on spring onions from the Racecourse market compared to the European market. For example, mean thermotolerant coliform numbers on spring onions from Racecourse market was 7.17 log units compared to the 4.45 log units recorded for samples from the European market (Table 12).

4.6 Effectiveness of sanitizers in decontaminating spring onions at the kitchen

Table 13 shows the initial and final thermotolerant coliform and helminth egg numbers after treating spring onions with vinegar or salt solutions. After treating spring onions with vinegar solution (100 ml of vinegar: 500 ml of water) for 10 minutes, there were

significant decrease (ρ = 0.000) in both thermotolerant coliforms and helminth egg numbers (Appendix C3 and C4).

Table 13: Thermotolerant coliform and helminth egg numbers on spring onions before and after treatment with vinegar or salt solution for 10 minutes

Types of Sanitizer	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E) [a]			(No. of Helminth eggs/100g sample)		
	Initial	Final	Reduction	Initial	Final	Reduction
Vinegar	6.09 (±0.14)	3.55 (±0.15)	2.54	2.4 (±0.34)	1.1 (±0.08)	1.3
Salt	6.09 (±0.14)	4.05 (±0.18)	2.01	2.4 (±0.34)	1.9 (±0.19)	0.5

Figures in parentheses represent the standard error (n=18 for each treatment)
Initial = pathogen level before use of sanitizer
Final = pathogen level after use of sanitizer
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean

Thermotolerant coliforms reduced from 6.09 to 3.55 log units while helminth egg numbers reduced from 2.4 to 1.1 per 100 g wet weight. When spring onion samples from the same stock were treated with salt solution (7 ppm) for 10 minutes, there were significant decrease (ρ = 0.000) in both thermotolerant coliforms and helminth egg numbers (Appendix C1 and C2). Thermotolerant coliforms reduced from 6.09 to 4.05 log units while helminth egg numbers were reduced from 2.4 to 1.9 per 100 g wet weight (Table 13).

4.7 Multiple barrier approach along production-consumption pathway of wastewater irrigated spring onions

Table 14 shows that cessation of irrigation for 2-days prior to harvesting reduced thermotolerant coliform numbers by 1.1 log units and 0.6 per 100 g wet weight of helminth eggs. The results also showed that when the same stock of spring onions were stored in basket, a reduction of 0.42 log units and 0.2 helminth eggs per 100 g wet weight were achieved for both thermotolerant coliforms and helminth eggs, respectively. Further reduction for thermotolerant coliforms (1.47 log units) and helminth eggs (0.4 per 100 g wet weight) were achieved when the bulbs of spring onion samples were washed in a bowl of water (8.5 litres) for 2 minutes and displayed in basket at the market for 2-3 hours. In addition, a reduction of 2.08 log units for thermotolerant coliforms and 1.0 per 100 g wet weight for helminth eggs were achieved after washing spring onions in vinegar solution (100 ml of vinegar: 500 ml of water) for a contact time of 10 minutes at the kitchen (Appendix D5). Statistically, a significant reduction ($\rho = 0.000$) for both thermotolerant coliforms and helminth egg numbers were achieved when spring onion samples were tracked from farm to the kitchen with vinegar as the main sanitizer used (Appendix D3 and D4).

Table 14: Effectiveness of various non-treatment interventions along the production-consumption pathway of wastewater irrigated spring onion

Intervention Assessment	Thermotolerant coliforms (Log_{10} MPN/100g ± S.E)			(No. of Helminth eggs/ 100 g sample)		
	Initial	Final	Reduction	Initial	Final	Log cycle Reduction
2-days cessation of irrigation prior to harvesting	5.54 (± 0.13)	4.44 (± 0.17)	1.1	2.2 (± 0.14)	1.6 (± 0.11)	0.6
Storage of spring onions overnight in baskets.	4.35 (±0.16)	3.93 (±0.23)	0.42	1.6 (± 0.11)	1.4 (±0.08)	0.2
Washing of bulbs (1 kg) in bowl water (8.5 litres) for 2 minutes and display in baskets at the market for two-three hours	3.98 (± 0.17)	2.51 (± 0.10)	1.47	1.4 (± 0.08)	1.0 (± 0.04)	0.4
Washing with vinegar (100 ml of vinegar:500 ml of water for 10 minutes	2.63 (± 0.09)	0.55 (± 0.05)	2.08	1.1 (± 0.07)	0.1 (± 0.03)	1.0
Washing with salt solution (7 ppm) for 10 minutes	2.63 (± 0.09)	0.62 (± 0.09)	2.01	1.1 (± 0.09)	0.8 (± 0.03)	0.3

Figures in parentheses represent the standard error (n=90)
Initial = pathogen level before intervention, Final = pathogen level after intervention
Values for thermotolerant coliform represent geometric mean and that of helminth eggs represent arithmetic mean

When spring onions were washed with salt solution (7 ppm) for 10 minutes, slightly lower numbers (2.01 log units) for thermotolerant coliforms and 0.3 per 100 g wet weight for helminth eggs were attained (Table 4.10). When the same stock of spring onions were tracked from farm to the kitchen with salt as the sanitizer, a significant decrease (ρ= 0.006) in thermotolerant coliforms and helminth egg (ρ = 0.001) numbers were recorded (Appendix D1 and D2).

4.8 Thermotolerant coliforms reduction on spring onions obtained from the study compared to WHO (2006) proposed standards for wastewater use in agriculture

From the study, thermotolerant coliform die off (2-day cessation of irrigation) was 0.55 log units per day and this fell within the range of WHO (2006) standard of 0.5-2 per day. Thermotolerant coliform reduction of 0.42 and 1.47 log unit were recorded for storage in basket and washing the bulb of spring onions in half bowl of water (8.5 litres) for two minutes and display in baskets at market. However, there are no proposed WHO (2006) standard for storage and washing at the market. Disinfection of spring onion with vinegar solution recorded 2.08 log unit reductions and this was above the proposed standard. Additionally, disinfection of spring onion with salt solution recorded 2.01 log unit reduction and this was slightly above the WHO (2006) proposed standard. A total reduction of 5.07 and 5.0 log units of thermotolerant coliform were achieved for vinegar and salt solution respectively. WHO (2006) proposes overall 6-7 log units reduction for bacteria (thermotolerant coliforms) which includes wastewater treatment (Appendix D5).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Quality of irrigation water at the production sites

This study showed that irrigation water used at 'Quarters' and 'D-line' production sites contain high numbers of thermotolerant coliforms and helminth eggs. At all the two sites, the mean thermotolerant coliforms and helminth egg numbers exceeded (WHO, 2006) guideline of ≤ 3 $\log_{10}$ /100ml of thermotolerant coliforms and ≤ 1 egg per litre for helminth eggs except 'D-line' farm which recorded levels within limits of the guideline for helminth. Based on this guideline, the wastewater used at 'Quarters' farm is unfit for use in agriculture.

At 'Quarters' farm, the source of irrigation water is shallow hand-dug well while that of 'D-line' is a stream. The higher incidence of contamination of irrigation water at 'Quarters' farm could be attributed to the heaps of poultry manure piles located uphill and close to the sources of the irrigation water. These piles could easily be washed into the irrigation water sources through runoffs. Studies by Drechsel *et al.* (2000) and Amoah *et al*. (2005) indicate that the location of poultry manure heaps allows for possible runoff into the irrigation water. Farm workers on the other hand, always step in the well to fetch the water for irrigation. This act stirs up the sediments in the water and causes settled helminth eggs to float on the surface which are easily fetched into the watering cans. At 'D-line' farm, farm workers stood at the bank of the stream to fetch the water and

therefore did not disturb the sediments much as it is the case of 'Quarters' farm. The result of this study is in agreement with reports made by Amoah *et al.* (2005) which indicated that shallow hand dug wells used for irrigating vegetables in Kumasi had higher faecal coliform counts compared to those of streams as hand dug wells are often not protected and easily receive pollutants from the surrounding environment through run off.

5.1.2 Effect of cessation time of irrigation on thermotolerant coliforms and helminth eggs on 100 g of wastewater irrigated spring onion

Results from the pre-test study, showed that irrigation stoppage prior to harvesting spring onions can effectively reduce microbial contamination during the dry season. An average daily reduction of 0.6 log unit for thermotolerant coliforms and 0.06 helminth eggs per 100 g wet weight of spring onions were achieved under field conditions. In very hot climates, higher reduction rates of up to 3 log units per day have been reported (Fattal *et al*., 2002). Work done in Ghana by Keraita *et al.* (2007a) also shows an average daily reduction of 0.65 log units for thermotolerant coliforms and 0.4 helminth eggs on 100 g for lettuce. However, recommended levels by WHO (2006) ranges between the range of 0.5 and 2.0 log unit reduction per day between final irrigation and consumption. The values recorded in this study for spring onions fall within the recommended levels. This could be due to the fact that as days of cessation of irrigation increases, the soil adhered to the bulbs of spring onion become dry and loose and is easily dislodged with the slightest agitation during harvesting. The daily pathogen reductions as a result of cessation of irrigation depends on climatic conditions, with more rapid pathogen die-off in hot, dry weather than in cool or wet weather conditions without much direct sunlight,

and the type of crop etc (WHO, 2006). Work done in Portugal during 1985-1989 (Vas da Costa *et al.*, 1996) explored the effect of the irrigation of salad crops with treated wastewater of various qualities. When poor quality trickling filter effluent (10 fecal coliforms (FC) per 100 ml) was used to spray-irrigate lettuces, the initial level of indicator bacteria on the lettuces (10^6 fecal coliform per 100 g) reflected the bacteriological quality of the irrigation water and exceeded the International Commission on Microbiological Specifications for Foods (ICMSF, 1974) recommendations ($<10^5$ FC per 100 g fresh weight, preferably $< 10^3$ FC per 100 g) for foodstuffs eaten raw. The study revealed that once irrigation ceased, fecal coliforms count were similar to the level seen in lettuces irrigated with fresh water. Similar work done in Ghana by Keraita *et al.* (2007a) also showed an average daily reduction of 0.65 log units for thermotolerant and 0.4 helminth eggs on 100 g lettuce. Helminth egg reduction on the other hand, was relatively low due to their highly resistant nature (Watson *et al*., 1999).

Prolonged cessation periods especially (four days and beyond) produced marked detrimental effects on productivity and freshness (overall visual quality) of spring onions. Insisting on cessation before harvest as a health risk reducing strategy may be difficult for leafy crops that need to be harvested at the peak of their freshness. Changes in the physical quality of spring onion were noticeable especially on the sixth day of cessation of irrigation. Freshness reduced drastically with most leaves turning brown and withering away. Loss in weight could be due to the fact that leaves of spring onions behave like inflated balloon upon irrigation. When irrigation stops for more than a day, the leave loses its turgidity, and results in loss in weight. During the sampling period, spring onion lost an average weight of 0.15 kg of fresh weight. Similar results were also reported in

Kumasi (Keraita *et al*., 2007a) when an average weight of 0.14 kg was lost by lettuce during cessation of irrigation in the dry season.

5.1.3 Farmers' perceptions on cessation of irrigation

Farmers, as part of maintaining the freshness of their produce, irrigate till harvesting. Farmers found cessation of irrigation for prolonged period especially beyond two days unacceptable. In an informal interview, they outlined the following as their main reasons for not wanting to embrace this concept: (1) Difficulty in harvesting; when irrigation is stopped for more than two days, the soil dries up and renders uprooting of spring onions difficult. They added that, if care is not taken, forcing to pull up the bunch of spring onion will result in the leaves tearing away from the bulb which then results in economic loss, (2) reduced freshness results from cessation of irrigation for more than two days. According to the farmers, "the leaves of spring onions are filled with air which makes it look like an inflated balloon. Prolonged cessation of irrigation takes away the air and therefore, undesirable effects, such as softening of the plant tissue may occur".

Irrespective of these difficulties, farmers saw this measure as one of the effective means of reducing contamination. Farmers were of the view that areas that are water logged could be used for prolong cessation of irrigation in the dry cessation as there will be constant water supply to meet the water requirement of the crop thereby sustaining the fresh weights.

Root crops such as onion are more prone to contamination and facilitate pathogen survival (WHO, 2006). In this study, an average difference of 2.67 log units of thermotolerant coliforms and 2.5 eggs per 100 g wet weight (Table 4.4) were found

between the bulb and leaves of spring onion. This is due to the fact that spring onion is a subterranean crop (Islam *et al.*, 2004) with the bulbs embedded in the soil and exposed to higher contamination load. He also reiterated that root crops have the greatest risk of contamination from manure and irrigation water application to soil. According to Beuchat (2002), surfaces of vegetable roots are characterized by unique microenvironments that influence colonization of bacteria, yeasts, and moulds. Similar study by Keraita *et al.* (2007a) recorded higher numbers of thermotolerant coliforms and helminth eggs on the outer leaves of lettuce that had more contact with soil than the inner leaves. The leaves of spring onions are slender (low surface area) with smooth surfaces and so cannot retain much water, and have very little soil on the surfaces. Previous studies by Stine *et al.* (2005) showed that lettuce and cantaloupe surfaces retained pathogens from irrigation water spiked with *E.coli* and a bacteriophage, but bell peppers which are smooth did not. Strauss, (1985) also concluded that there is lower die-off of faecal organisms in soil than on exposed crop surfaces. Finally, Moore *et al.* (2006) emphasise that pathogens survive on crop surfaces for a shorter time than in the soil as they are less well protected from the harsh effects of sunlight and desiccation.

5.1.4 Management of watering can irrigation to reduce thermotolerant coliforms and helminth egg numbers on wastewater irrigated spring onion

Irrigating of spring onions from different heights with or without perforated caps at the outlet also has significant influence on contamination. A recent study carried out by Keraita *et al.* (2007b), showed that increasing watering heights when using watering cans whether perforated or not, increased both thermotolerant coliforms and helminth eggs on

lettuce significantly. The results obtained for spring onions were contrary to results obtained by Keraita (2007b). Decreasing watering heights, whether watering can was perforated at the outlet or not rather increased thermotolerant coliforms significantly. This differing result could be due to more soil wetting leading to the bulbs holding a lot of soil. A study by Feachem *et al.* (1998) states that contact with wastewater irrigated soil, where E.*coli* and in particular helminth eggs can survive for prolong periods is a potential risk factor for pathogen transmission. Buck *et al*. (2003) also states that sources of microbial pathogens on fresh produce at the preharvest stage include soil, faeces, irrigation water, inadequately composted manure, animals etc. With helminth eggs, there was no significant difference between the treatments. Once there is application of water, soil become wet and there is the tendency of soil (which might accumulate helminth eggs) adhering to the bulb. Helminth eggs are known to be very "sticky", so they easily adhere to crop surfaces (WHO, 2006) upon watering.

5.2 Post-harvest handling practices and effectiveness of proposed non-treatment interventions at market study site

Food markets have an essential function of providing consumers with safe and nutritious food (WHO, 2006). Though wastewater has been pointed out as the major contributor to contamination of vegetables, there is increasing evidence of recontamination of vegetables, as it is transported from the farm gate to the markets, to the food vendors or kitchen and finally to consumers. Contamination events occurring during post-harvest stages involved storage after harvesting, harvesting equipment, transport vehicles, and other human handling practices in markets (Beuchat, 1995).

An informal interview carried out at the Racecourse market where bulk of the data were collected revealed that, spring onions are generally harvested the previous day by market women and stored in sacks. This aimed at targeting marketers who arrive early in the morning to buy the vegetables. Spring onions stored in baskets had lower thermotolerant coliform and helminth egg numbers than those stored in sacks and freshly harvested (Table 9). Basket as a storage vessel has a lot of holes in it which will allow soil particles to be easily dislodged with the least agitation. According to Boyhan *et al*. (1999), the key to preserving onions and to prevent bruising is to keep them cool; dry and separated. Studies carried out by (Gast and Flores, 2004) also showed that proper storage conditions (low temperature and humidity) are needed to lengthen storage life and maintain quality. He stated that low temperatures and humidity slow the growth of pathogenic microorganisms which cause spoilage of fruits and vegetables in storage. From the current study, storage of spring onions overnight in basket had lower thermotolerant coliforms and helminth egg numbers. However, there was no difference in terms of visual ratings between those stored in basket and in the sack. Allende *et al*. (2007) stated that neither the level of total count nor the level of specific spoilage microorganisms per se can directly predict the sensory quality of a product.

Spring onion stored overnight, are transported to the market at dawn for sale. In most markets, spring onions are displayed for sale in baskets, bowls and on tables (Table 10). Displaying points in the market do not have much influence on contamination levels. This could be attributed to the fact that, spring onions are displayed without much additional processing such as repackaging and washing as it is in the case of lettuce (Obuobie *et al.*, 2006).

An informal interview with the vegetable sellers during the study period revealed that, washing reduces firmness and enhances browning of leaves as well. Nevertheless, different washing practices were carried out in the market to ascertain the truth that washing affects produce quality and also to determine which mode of washing could best reduce contamination without loss to produce quality. As expected washing of spring onions (whole plant) under running tap water for two minutes proved to be the best method of reducing contamination rather than washing in bowl of water (8.5 litres) for the same period. Washing spring onions for two minutes under running tap water recorded 4.75 log units reduction of thermotolerant coliforms. This agrees with studies carried out by Amoah *et al*. (2007), who found that washing of lettuce under running tap without even a sanitizer is effective in reducing faecal contamination loads. They further stated that, washing lettuce for 2 min under running tap water achieved the highest reduction of 2.2 log units.

The report that quality of leaves such as firmness and colour are reduced after washing was confirmed in this study. Leaves of washed spring onions looked flaccid and pale compared to the unwashed ones which were turgid and green. In view of this, washing only the bulbs of spring onions which mostly harbour a lot of contamination (Table 11) was also tested. Again, washing only bulbs of spring onions under running tap was more effective than washing in a bowl of water (8.5 litres). Traders were of the view that, if there must be washing of spring onions in the market it should be the washing of the bulbs which holds soil particles in a bowl of water and not the leaves.

This study further assessed how many times the bulbs of spring onions could be washed in a specific amount of water to effectively reduce microbial contamination and

also minimise cross contamination. Washing of bulbs of spring onions in the same amount of water continuously could result in cross-contamination. After the third washing cycle, there was gradual increase of thermotolerant coliforms and helminth egg numbers on bulbs of spring onion (Fig 4.1). This resulted in decreasing efficacy after washing a total of 5 kg bulbs of spring onions (in five cycles of 1 kg per cycle) in the same bowl of water.

Different handling practices at various markets also influence contamination levels. European market which is patronized by high income earning people within the metropolis had the lowest level of contamination (Table 12) due to better handling practices. The practices include washing the bulbs of spring onions in a bowl of water before they are displayed for sale. European market is often patronized by foreigners, and as such special care is taken to present the vegetable in the most attractive ways. On the other hand in the other market, vegetables are displayed without any form of pre-treatment.

5.3 Effectiveness of sanitizers

Selected sanitizers for this study was based on recommendations made by Amoah *et al*. (2007), who suggested that lower concentrations of vinegar (approximately 100 ml of vinegar: 500 ml of water) and salt solution (7 ppm) with contact time of 10 minutes could be effective. From this study, a reduction of 2.54 and 2.04 log units thermotolerant coliforms were achieved for vinegar and salt solution, respectively. Helminth egg reductions were 1.3 for vinegar and 0.5 for salt solution. Amoah *et al*. (2007) also obtained 2.83 log units reduction for faecal coliforms when lettuce were treated with

vinegar. Data from this work confirmed that vinegar is more effective than salt. However, despite the difference in the effectiveness of these sanitizers, levels obtained were higher compared to the recommended levels in the WHO (2006) guideline. This could be due to peeling off sheath covering the bulb of spring onions before washing in a sanitizer. This peeling of root crop has been proposed by the WHO (2006) guideline to give a pathogen log reduction of 2 units.

Even though there are no clear cut reduction levels for helminth eggs in the WHO (2006) guidelines for the different post harvest health protection measures as it is for thermotolerant coliforms (Appendix D5), it states that washing of crops in a weak detergent solution and rinsing thoroughly with safe drinking water can remove helminth eggs from surface of crops eaten raw. This is confirmed by the helminth egg reductions of 1.3 and 0.5 per 100 g wet weight achieved in the study when spring onions were washed with vinegar and salt sanitizers, respectively. According to Suslow (2000), the use of sanitizing agents in water is essential to kill microbes before they can attach or become internalized in produce. Generally, it is accepted that an ideal sanitizing agent should have two important properties. It should have a sufficient level of antimicrobial activity and a negligible effect on the sensory quality of the product (Allende *et al.*, 2007). A lower concentration of 100 ml of vinegar: 500 ml of water combined with an increase in contact time (up to 10 minutes) could be equally effective (Amoah *et al.*, 2007). These higher efficiencies could be related to both higher oxidizing capacity or higher surfactant activity, which allows better contact between attached bacteria and the active compound of the sanitizers (Sapers, 2001). However it should be taken into account that the concentration of sanitizers or other chemical-based intervention methods

may be limited by unacceptable sensory impact on the produce (Brackett, 1992). Therefore, the sensory quality should be also evaluated when selecting the optimal sanitizing technique (Mart´inez-S´anchez and Murcia, 2006).

The work has demonstrated that, all the criteria tested above can be effective only if used as part of a multiple barrier approach in reducing contamination on wastewater irrigated spring onions.

5.4 Cumulative pathogen reductions achieved for spring onions through multiple barrier approach along the production-consumption pathway

WHO (2006) recommends a performance target of 6-7 log units reduction in order to meet the health based target of a tolerable additional burden of disease from wastewater use of $\leq 10^6$ disability adjusted life years (DALY) per person per year. This performance target can easily be achieved by effective wastewater treatment using high-technology tertiary treatments and disinfection systems (WHO, 2006). Treatment technology used in industrialized countries tends to be unsustainable in the developing countries partly because of the high cost associated with their use. Therefore treatment or guidelines cannot be solely relied upon. A number of strategies which will collectively protect people's health and livelihoods should be concurrently applied. The study therefore focused on reducing contamination on the crop after wastewater has been applied. A combination of locally feasible non-treatment options from production site ('Quarters' farm) to kitchen was assessed for their effectiveness. One production site was selected for the tracking process since it presented higher risk potential than 'D-line' farm. The barriers selected included two-day cessation of irrigation (D2), storage of spring onions

in baskets overnight, washing bulbs of spring onions and displaying in basket at the market and treatment spring onions with vinegar or salt solution at kitchen.

A total of 6-7 pathogen reduction may be achieved by the application of appropriate health protection measures, each of which has its own associated log unit reduction or range of log unit reductions. The sum of the individual log unit reduction for each post-treatment health protection measure assessed in the study equal to 5.07 (vinegar) and 5.0 (salt solution) log units for thermotolerant coliforms with 2.2 (vinegar) and 1.5 (salt solution) helminth eggs for vinegar and salt solution, respectively (Appendix D5). Complementary barriers to interrupt the flow of pathogen at all identified entry points in the production-consumer pathway even without wastewater treatment will provide a greater margin of food safety. Beuchat (1998) concluded from his studies that prevention of contamination at all points of the food chain is highly preferred.

5.5 CONCLUSION

This study has shown that a combination of different non-treatment interventions from farm to kitchen can be used to interrupt the flow of pathogens thereby reducing thermotolerant coliforms and helminth egg numbers on wastewater irrigated spring onions. On the farm, cessation of irrigation for two days prior to harvesting spring onions reduced thermotolerant coliforms significantly but not helminth egg numbers. Pathogen reduction was also achieved when spring onions were stored in baskets for transportation to market. Good washing practices can best reduce contamination in the market. However, washing affects the firmness of the leaves which consequently reduce its marketability. Therefore, bulbs of spring onions where pockets of soil are found should

be washed. Disinfecting procedures of the raw eaten salad vegetables like spring onions can also help reduce contamination at the kitchen level. These practical steps can be taken in the short and medium terms to reduce adverse health impacts associated with raw wastewater for crop irrigation.

5.6 RECOMMENDATIONS

The maintenance of good public relations, especially with respect to protection of consumer health is a very important task. Consumers must have confidence that the vegetables (spring onions) they are eating are not injurious to their health. In this respect, programmes for the routine monitoring of wastewater use and produce quality are thus key to protecting farmers and consumers safety. It is therefore recommended that:

1. Effective on-farm hygiene promotion programmes such as standing at the edge of ponds to fetch water gently without disturbing the sediments be organized for farm workers regularly;
2. Poultry manure should be stored down hill to avoid run-off into the source of irrigation water;
3. Extra drinking water posts should be provided in the markets to enable vegetable sellers have easy access to potable water for washing their produce;
4. Crops should be irrigated with non-contaminated water sources (where available) after cessation of wastewater irrigation until harvest;
5. Spring onions could be stored in baskets instead of sacks;
6. Bulbs of spring onions should be washed in the market to remove contaminated soil before it enters the kitchen;

7. Local food handlers should be educated to wash wastewater-irrigated produce effectively with water or disinfectant solutions (salt and vinegar) and,
8. Further research should be conducted to quantify the risk of pathogen exposure for all stakeholders along the producer-consumer pathway through Quantitative Microbial Risk Assessment (QMRA) model to ensure conclusive evidence of disease transmission.

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APPENDIX A

T-Test and ANOVA results at production sites

A1- T- test for Thermotolerant numbers in irrigation water at Quarters and D-line farms

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Log Thermotolerant coliforms	Equal variances assumed	.078	.783	3.470	14	.004	1.13474	.32705	.43330	1.83619
	Equal variances not assumed			3.470	13.945	.004	1.13474	.32705	.43304	1.83645

A2- T- test for Helminth egg numbers in irrigation water at Quarters and D-line farms

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Log Helminth eggs	Equal variances assumed	4.103	.062	2.835	14	.013	.71667	.25276	.17454	1.25879
	Equal variances not assumed			2.835	11.750	.015	.71667	.25276	.16464	1.26870

ANOVA results before and after assessing non treatment interventions at production sites

A3- Anova for (0-6days) cessation of irrigation at Quarters farm

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	70.074	3	23.358	16.966	.000
	Within Groups	49.564	36	1.377		
	Total	119.639	39			
Log Helminth eggs	Between Groups	.851	3	.284	.920	.441
	Within Groups	11.092	36	.308		
	Total	11.943	39			

A3i- Multiple comparison for (0-6 days) cessation of irrigation at 'Quarters' farm

Dependent Variable: Log Thermotolerant coliforms

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Irrigating till harvesting	Cessation of irrigation 2-days before harvesting	1.14154*	.53655	.041	.0499	2.2332
	Cessation of irrigation 4-days before harvesting	2.30207*	.53655	.000	1.2104	3.3937
	Cessation of irrigation 6-days before harvesting	3.83574*	.59125	.000	2.6328	5.0387
Cessation of irrigation 2-days before harvesting	Irrigating till harvesting	-1.14154*	.53655	.041	-2.2332	-.0499
	Cessation of irrigation 4-days before harvesting	1.16053*	.53655	.038	.0689	2.2522
	Cessation of irrigation 6-days before harvesting	2.69420*	.59125	.000	1.4913	3.8971
Cessation of irrigation 4-days before harvesting	Irrigating till harvesting	-2.30207*	.53655	.000	-3.3937	-1.2104
	Cessation of irrigation 2-days before harvesting	-1.16053*	.53655	.038	-2.2522	-.0689
	Cessation of irrigation 6-days before harvesting	1.53368*	.59125	.014	.3308	2.7366
Cessation of irrigation 6-days before harvesting	Irrigating till harvesting	-3.83574*	.59125	.000	-5.0387	-2.6328
	Cessation of irrigation 2-days before harvesting	-2.69420*	.59125	.000	-3.8971	-1.4913
	Cessation of irrigation 4-days before harvesting	-1.53368*	.59125	.014	-2.7366	-.3308

*. The mean difference is significant at the .05 level.

A3ii- Multiple comparisons for (0-6 days) cessation of irrigation at 'Quarters' farm

Dependent Variable: Log Helminth eggs

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Irrigating till harvesting	Cessation of irrigation 2-days before harvesting	.20250	.24826	.420	-.3010	.7060
	Cessation of irrigation 4-days before harvesting	.32571	.24826	.198	-.1778	.8292
	Cessation of irrigation 6-days before harvesting	.37917	.24826	.135	-.1243	.8827
Cessation of irrigation 2-days before harvesting	Irrigating till harvesting	-.20250	.24826	.420	-.7060	.3010
	Cessation of irrigation 4-days before harvesting	.12321	.24826	.623	-.3803	.6267
	Cessation of irrigation 6-days before harvesting	.17667	.24826	.481	-.3268	.6802
Cessation of irrigation 4-days before harvesting	Irrigating till harvesting	-.32571	.24826	.198	-.8292	.1778
	Cessation of irrigation 2-days before harvesting	-.12321	.24826	.623	-.6267	.3803
	Cessation of irrigation 6-days before harvesting	.05345	.24826	.831	-.4500	.5569
Cessation of irrigation 6-days before harvesting	Irrigating till harvesting	-.37917	.24826	.135	-.8827	.1243
	Cessation of irrigation 2-days before harvesting	-.17667	.24826	.481	-.6802	.3268
	Cessation of irrigation 4-days before harvesting	-.05345	.24826	.831	-.5569	.4500

A4- Anova for fresh weights of spring onions after (0-6 days) cessation of irrigation at 'Quarters' farm

Fresh weights

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.768	3	1.589	14.669	.000
Within Groups	3.901	36	.108		
Total	8.669	39			

A4i-Multiple comparisons for fresh weights of spring onions after (0-6 days) cessation of irrigation at Quarters farm

Dependent Variable: Fresh weights

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Fresh weights for irrigating till harvesting	Fresh weights after 2-days cessation of irrigation	.31200*	.14721	.041	.0135	.6105
	Fresh weights after 4-days cessation of irrigation	.62200*	.14721	.000	.3235	.9205
	Fresh weights after 6-days cessation of irrigation	.92600*	.14721	.000	.6275	1.2245
Fresh weights after 2-days cessation of irrigation	Fresh weights for irrigating till harvesting	-.31200*	.14721	.041	-.6105	-.0135
	Fresh weights after 4-days cessation of irrigation	.31000*	.14721	.042	.0115	.6085
	Fresh weights after 6-days cessation of irrigation	.61400*	.14721	.000	.3155	.9125
Fresh weights after 4-days cessation of irrigation	Fresh weights for irrigating till harvesting	-.62200*	.14721	.000	-.9205	-.3235
	Fresh weights after 2-days cessation of irrigation	-.31000*	.14721	.042	-.6085	-.0115
	Fresh weights after 6-days cessation of irrigation	.30400*	.14721	.046	.0055	.6025
Fresh weights after 6-days cessation of irrigation	Fresh weights for irrigating till harvesting	-.92600*	.14721	.000	-1.2245	-.6275
	Fresh weights after 2-days cessation of irrigation	-.61400*	.14721	.000	-.9125	-.3155
	Fresh weights after 4-days cessation of irrigation	-.30400*	.14721	.046	-.6025	-.0055

*. The mean difference is significant at the .05 level.

A5-ANOVA for (0-6 days) cessation of irrigation at 'D-line' farm

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	41.130	3	13.710	15.074	.000
	Within Groups	29.104	32	.909		
	Total	70.234	35			
Log Helminth eggs	Between Groups	.588	3	.196	.856	.474
	Within Groups	7.326	32	.229		
	Total	7.914	35			

A5i-Multiple comparisons for (0-6days) cessation of irrigation at 'D-line' farm

Dependent Variable: Log Thermotolerant coliforms

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Irrigating till harvesting	Cessation of irrigation 2-days before harvesting	1.00290*	.44957	.033	.0872	1.9186
	Cessation of irrigation 4-days before harvesting	1.91761*	.44957	.000	1.0019	2.8333
	Cessation of irrigation 6-days before harvesting	2.88141*	.44957	.000	1.9657	3.7971
Cessation of irrigation 2-days before harvesting	Irrigating till harvesting	-1.00290*	.44957	.033	-1.9186	-.0872
	Cessation of irrigation 4-days before harvesting	.91471	.44957	.050	-.0010	1.8304
	Cessation of irrigation 6-days before harvesting	1.87851*	.44957	.000	.9628	2.7942
Cessation of irrigation 4-days before harvesting	Irrigating till harvesting	-1.91761*	.44957	.000	-2.8333	-1.0019
	Cessation of irrigation 2-days before harvesting	-.91471	.44957	.050	-1.8304	.0010
	Cessation of irrigation 6-days before harvesting	.96380*	.44957	.040	.0481	1.8795
Cessation of irrigation 6-days before harvesting	Irrigating till harvesting	-2.88141*	.44957	.000	-3.7971	-1.9657
	Cessation of irrigation 2-days before harvesting	-1.87851*	.44957	.000	-2.7942	-.9628
	Cessation of irrigation 4-days before harvesting	-.96380*	.44957	.040	-1.8795	-.0481

*. The mean difference is significant at the .05 level.

A5ii- Multiple comparisons for (0-6 days) cessation of irrigation at 'D-line' farm

Dependent Variable: Log helminth eggs

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Irrigating till harvesting	Cessation of irrigation 2-days before harvesting	.19444	.22555	.395	-.2650	.6539
	Cessation of irrigation 4-days before harvesting	.28704	.22555	.212	-.1724	.7465
	Cessation of irrigation 6-days before harvesting	.33333	.22555	.149	-.1261	.7928
Cessation of irrigation 2-days before harvesting	Irrigating till harvesting	-.19444	.22555	.395	-.6539	.2650
	Cessation of irrigation 4-days before harvesting	.09259	.22555	.684	-.3668	.5520
	Cessation of irrigation 6-days before harvesting	.13889	.22555	.542	-.3205	.5983
Cessation of irrigation 4-days before harvesting	Irrigating till harvesting	-.28704	.22555	.212	-.7465	.1724
	Cessation of irrigation 2-days before harvesting	-.09259	.22555	.684	-.5520	.3668
	Cessation of irrigation 6-days before harvesting	.04630	.22555	.839	-.4131	.5057
Cessation of irrigation 6-days before harvesting	Irrigating till harvesting	-.33333	.22555	.149	-.7928	.1261
	Cessation of irrigation 2-days before harvesting	-.13889	.22555	.542	-.5983	.3205
	Cessation of irrigation 4-days before harvesting	-.04630	.22555	.839	-.5057	.4131

\6- ANOVA for fresh weights of spring onions after (0-6 days) cessation of irrigation at D-line farm

frshwght

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.703	3	.568	160.659	.000
Within Groups	.127	36	.004		
Total	1.830	39			

A6i- Multiple Comparisons for fresh weight of spring onions after (0-6 days) cessation of irrigation at 'D-line' farms

Dependent Variable: Fresh weights

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Fresh weight for iriigation till harvestiong	Fresh weights after 2-days cessation of irrigation	.50000*	.04249	.000	.4134	.5866
	Fresh weiths after 4- days cessation of irrigation	.75000*	.04249	.000	.6634	.8366
	Fresh weights after 6-days cessation of irrigation	1.21667*	.04249	.000	1.1301	1.3032
Fresh weights after 2-days cessation of irrigation	Fresh weight for iriigation till harvestiong	-.50000*	.04249	.000	-.5866	-.4134
	Fresh weiths after 4- days cessation of irrigation	.25000*	.04249	.000	.1634	.3366
	Fresh weights after 6-days cessation of irrigation	.71667*	.04249	.000	.6301	.8032
Fresh weiths after 4- days cessation of irrigation	Fresh weight for iriigation till harvestiong	-.75000*	.04249	.000	-.8366	-.6634
	Fresh weights after 2-days cessation of irrigation	-.25000*	.04249	.000	-.3366	-.1634
	Fresh weights after 6-days cessation of irrigation	.46667*	.04249	.000	.3801	.5532
Fresh weights after 6-days cessation of irrigation	Fresh weight for iriigation till harvestiong	-1.21667*	.04249	.000	-1.3032	-1.1301
	Fresh weights after 2-days cessation of irrigation	-.71667*	.04249	.000	-.8032	-.6301
	Fresh weiths after 4- days cessation of irrigation	-.46667*	.04249	.000	-.5532	-.3801

*. The mean difference is significant at the .05 level.

A7 T-Test for leaves and bulbs of spring onions

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
LogTTC	Equal variances assumed	4.112	.070	-9.315	10	.000	-2.68653	.28840	-3.32912	-2.04394
	Equal variances not assumed			-9.315	6.583	.000	-2.68653	.28840	-3.37734	-1.99571
LogHEL	Equal variances assumed	.165	.694	-5.276	10	.000	-1.62500	.30798	-2.31121	-.93879
	Equal variances not assumed			-5.276	9.540	.000	-1.62500	.30798	-2.31573	-.93427

A8- ANOVA for watering cans with perforated caps at the outlets at heights <0.5, 0.75 and > 1.0 m

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliform	Between Groups	5.614	2	2.807	9.397	.001
	Within Groups	9.857	33	.299		
	Total	15.471	35			
Log Helminth eggs	Between Groups	1.717	2	.859	2.004	.151
	Within Groups	14.140	33	.428		
	Total	15.857	35			

A8i- Multiple Comparisons for watering cans with perforated caps at the outlet at heights < 0.5,0.75 and 0.75 r

Dependent Variable: Log Thermotolerant coliform

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Watering with perforated caps at height < 0.5 m	Watering with perforated caps at heights of about 0.75 m	.67038*	.22312	.005	.2164	1.1243
	Watering with perforated caps at heights > 1.0 m	.93910*	.22312	.000	.4851	1.3930
Watering with perforated caps at heights of about 0.75 m	Watering with perforated caps at height < 0.5 m	-.67038*	.22312	.005	-1.1243	-.2164
	Watering with perforated caps at heights > 1.0 m	.26872	.22312	.237	-.1852	.7227
Watering with perforated caps at heights > 1.0 m	Watering with perforated caps at height < 0.5 m	-.93910*	.22312	.000	-1.3930	-.4851
	Watering with perforated caps at heights of about 0.75 m	-.26872	.22312	.237	-.7227	.1852

*. The mean difference is significant at the .05 level.

A8ii- Multiple Comparisons for watering cans with perforated caps at the outlet of heights < 0.5,0.75 and <1.0 m

Dependent Variable: Log Helminth egss

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Watering with perforated caps at height < 0.5 m	Watering with perforated caps at about 0.75 m high	.25347	.26723	.350	-.2902	.7972
	Watering wuth perforated caps at heights > 1.0 m	.53472	.26723	.054	-.0090	1.0784
Watering with perforated caps at about 0.75 m high	Watering with perforated caps at height < 0.5 m	-.25347	.26723	.350	-.7972	.2902
	Watering wuth perforated caps at heights > 1.0 m	.28125	.26723	.300	-.2624	.8249
Watering wuth perforated caps at heights > 1.0 m	Watering with perforated caps at height < 0.5 m	-.53472	.26723	.054	-1.0784	.0090
	Watering with perforated caps at about 0.75 m high	-.28125	.26723	.300	-.8249	.2624

A9- ANOVA for watering cans without perforated caps at the outlet of heights < 0.5, 0.75 and 1.0 m

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliform	Between Groups	6.414	2	3.207	10.272	.000
	Within Groups	10.303	33	.312		
	Total	16.717	35			
Log Helminth eggs	Between Groups	.081	2	.041	.291	.750
	Within Groups	4.605	33	.140		
	Total	4.686	35			

A9i-Multiple Comparisons for watering cans without perforated caps at the outlet of heights < 0.5,0.75 and > 1.0 m

LSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Log Thermotolerant coliform	Watering without perforated caps at height < 0.5 m	Watering without perforated caps at heights of about 0.75 m	.79261*	.22811	.001	.3285	1.2567
		Watering without perforated caps at heights > 1.0 m	.97125*	.22811	.000	.5072	1.4353
	Watering without perforated caps at heights of about 0.75 m	Watering without perforated caps at height < 0.5 m	-.79261*	.22811	.001	-1.2567	-.3285
		Watering without perforated caps at heights > 1.0 m	.17864	.22811	.439	-.2855	.6427
	Watering without perforated caps at heights > 1.0 m	Watering without perforated caps at height < 0.5 m	-.97125*	.22811	.000	-1.4353	-.5072
		Watering without perforated caps at heights of about 0.75 m	-.17864	.22811	.439	-.6427	.2855
Log Helminth eggs	Watering without perforated caps at height < 0.5 m	Watering without perforated caps at heights of about 0.75 m	-.025	.152	.873	-.33	.29
		Watering without perforated caps at heights > 1.0 m	.086	.152	.576	-.22	.40
	Watering without perforated caps at heights of about 0.75 m	Watering without perforated caps at height < 0.5 m	.025	.152	.873	-.29	.33
		Watering without perforated caps at heights > 1.0 m	.111	.152	.473	-.20	.42
	Watering without perforated caps at heights > 1.0 m	Watering without perforated caps at height < 0.5 m	-.086	.152	.576	-.40	.22
		Watering without perforated caps at heights of about 0.75 m	-.111	.152	.473	-.42	.20

*. The mean difference is significant at the .05 level.

APPENDIX B

ANOVA results before and after assessment of non-treatment interventions at the market

B1- ANOVA for storage of spring onions

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	23.368	2	11.684	32.133	.000
	Within Groups	11.999	33	.364		
	Total	35.368	35			
Log Helminth eggs	Between Groups	2.427	2	1.213	47.057	.000
	Within Groups	.851	33	.026		
	Total	3.277	35			

B1i- Multiple comparisons for storage of spring onions

LSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Log Thermotolerant Coliforms	Freshly harvested	Storage in basket	1.75862*	.24618	.000	1.2578	2.2595
		Storage in sack	.10375	.24618	.676	-.3971	.6046
	Storage in basket	Freshly harvested	-1.75862*	.24618	.000	-2.2595	-1.2578
		Storage in sack	-1.65487*	.24618	.000	-2.1557	-1.1540
	Storage in sack	Freshly harvested	-.10375	.24618	.676	-.6046	.3971
		Storage in basket	1.65487*	.24618	.000	1.1540	2.1557
Log Helminth eggs	Freshly harvested	Storage in basket	.54914*	.06555	.000	.4158	.6825
		Storage in sack	-.00319	.06555	.961	-.1366	.1302
	Storage in basket	Freshly harvested	-.54914*	.06555	.000	-.6825	-.4158
		Storage in sack	-.55234*	.06555	.000	-.6857	-.4190
	Storage in sack	Freshly harvested	.00319	.06555	.961	-.1302	.1366
		Storage in basket	.55234*	.06555	.000	.4190	.6857

*. The mean difference is significant at the .05 level.

B2-ANOVA for displaying points(under tables, on tables, in bowls and baskets)

		Sum of Squares	df	Mean Square	F	Sig.
Log thermotolerant coliforms	Between Groups	.204	2	.102	.236	.791
	Within Groups	14.273	33	.433		
	Total	14.477	35			
Log Helminths	Between Groups	4.962	2	2.481	2.426	.104
	Within Groups	33.750	33	1.023		
	Total	38.712	35			

B2-ANOVA for displaying points (under tables, on tables, in bowls and baskts

		Sum of Squares	df	Mean Square	F	Sig.
Log thermotolerant coliforms	Between Groups	.204	2	.102	.236	.791
	Within Groups	14.273	33	.433		
	Total	14.477	35			
Log Helminths	Between Groups	4.962	2	2.481	2.426	.104
	Within Groups	33.750	33	1.023		
	Total	38.712	35			

B3- ANOVA for different washing practices of spring onions (whole plant)

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	146.701	2	73.351	182.202	.000
	Within Groups	13.285	33	.403		
	Total	159.986	35			
Log Helminth eggs	Between Groups	65.603	2	32.801	16.621	.000
	Within Groups	65.124	33	1.973		
	Total	130.727	35			

B3i- Multiple comparisons for different washing practices of spring onions (whole plant)

LSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Log Thermotolerant coliforms	Washing whole plant spring onions in a bowl	Washing whole plant spring onion under running tap	.90906*	.25903	.001	.3821	1.4361
		Unwashed whole plant spring onion	-3.75473*	.25903	.000	-4.2817	-3.2277
	Washing whole plant spring onion under running tap	Washing whole plant spring onions in a bowl	-.90906*	.25903	.001	-1.4361	-.3821
		Unwashed whole plant spring onion	-4.66379*	.25903	.000	-5.1908	-4.1368
	Unwashed whole plant spring onion	Washing whole plant spring onions in a bowl	3.75473*	.25903	.000	3.2277	4.2817
		Washing whole plant spring onion under running tap	4.66379*	.25903	.000	4.1368	5.1908
Log Helminth eggs	Washing whole plant spring onions in a bowl	Washing whole plant spring onion under running tap	1.41667*	.57351	.019	.2499	2.5835
		Unwashed whole plant spring onion	-1.87917*	.57351	.002	-3.0460	-.7124
	Washing whole plant spring onion under running tap	Washing whole plant spring onions in a bowl	-1.41667*	.57351	.019	-2.5835	-.2499
		Unwashed whole plant spring onion	-3.29583*	.57351	.000	-4.4626	-2.1290
	Unwashed whole plant spring onion	Washing whole plant spring onions in a bowl	1.87917*	.57351	.002	.7124	3.0460
		Washing whole plant spring onion under running tap	3.29583*	.57351	.000	2.1290	4.4626

*. The mean difference is significant at the .05 level.

B4- ANOVA for different washing practices of spring onions (bulbs)

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	66.280	2	33.140	87.654	.000
	Within Groups	12.476	33	.378		
	Total	78.756	35			
Log Helminth eggs	Between Groups	64.764	2	32.382	74.700	.000
	Within Groups	14.305	33	.433		
	Total	79.070	35			

B4i- Multiple comparisons for different washing practices of spring onions (bulbs)

LSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Log Thermotolerant coliforms	Washing whole plant spring onions in a bowl	Washing whole plant spring onion under running tap	.82302*	.25102	.002	.3123	1.3337
		Unwashed whole plant spring onion	-2.37720*	.25102	.000	-2.8879	-1.8665
	Washing whole plant spring onion under running tap	Washing whole plant spring onions in a bowl	-.82302*	.25102	.002	-1.3337	-.3123
		Unwashed whole plant spring onion	-3.20022*	.25102	.000	-3.7109	-2.6895
	Unwashed whole plant spring onion	Washing whole plant spring onions in a bowl	2.37720*	.25102	.000	1.8665	2.8879
		Washing whole plant spring onion under running tap	3.20022*	.25102	.000	2.6895	3.7109
Log Helminth eggs	Washing whole plant spring onions in a bowl	Washing whole plant spring onion under running tap	-2.40972*	.26879	.000	-2.9566	-1.8629
		Unwashed whole plant spring onion	.72917*	.26879	.011	.1823	1.2760
	Washing whole plant spring onion under running tap	Washing whole plant spring onions in a bowl	2.40972*	.26879	.000	1.8629	2.9566
		Unwashed whole plant spring onion	3.13889*	.26879	.000	2.5920	3.6858
	Unwashed whole plant spring onion	Washing whole plant spring onions in a bowl	-.72917*	.26879	.011	-1.2760	-.1823
		Washing whole plant spring onion under running tap	-3.13889*	.26879	.000	-3.6858	-2.5920

*. The mean difference is significant at the .05 level.

B5- ANOVA for washing cycles of sprin onion bulbs

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	48.395	5	9.679	32.954	.000
	Within Groups	14.098	48	.294		
	Total	62.494	53			
Log Helminth eggs	Between Groups	1.300	5	.260	2.367	.053
	Within Groups	5.275	48	.110		
	Total	6.575	53			

B5I- Multiple comparisons for washing cycles (Thermotolerant coliforms) of spring onions-Bulbs

Dependent Variable: Log Thermotolerant coliforms

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
No washing	Cycle 1	2.11719*	.25548	.000	1.6035	2.6309
	Cycle 2	2.07037*	.25548	.000	1.5567	2.5840
	Cycle 3	1.70973*	.25548	.000	1.1961	2.2234
	Cycle 4	.69828*	.25548	.009	.1846	1.2120
	Cycle 5	-.19001	.25548	.461	-.7037	.3237
Cycle 1	No washing	-2.11719*	.25548	.000	-2.6309	-1.6035
	Cycle 2	-.04682	.25548	.855	-.5605	.4668
	Cycle 3	-.40746	.25548	.117	-.9211	.1062
	Cycle 4	-1.41891*	.25548	.000	-1.9326	-.9052
	Cycle 5	-2.30720*	.25548	.000	-2.8209	-1.7935
Cycle 2	No washing	-2.07037*	.25548	.000	-2.5840	-1.5567
	Cycle 1	.04682	.25548	.855	-.4668	.5605
	Cycle 3	-.36064	.25548	.165	-.8743	.1530
	Cycle 4	-1.37209*	.25548	.000	-1.8858	-.8584
	Cycle 5	-2.26037*	.25548	.000	-2.7740	-1.7467
Cycle 3	No washing	-1.70973*	.25548	.000	-2.2234	-1.1961
	Cycle 1	.40746	.25548	.117	-.1062	.9211
	Cycle 2	.36064	.25548	.165	-.1530	.8743
	Cycle 4	-1.01145*	.25548	.000	-1.5251	-.4978
	Cycle 5	-1.89974*	.25548	.000	-2.4134	-1.3861
Cycle 4	No washing	-.69828*	.25548	.009	-1.2120	-.1846
	Cycle 1	1.41891*	.25548	.000	.9052	1.9326
	Cycle 2	1.37209*	.25548	.000	.8584	1.8858
	Cycle 3	1.01145*	.25548	.000	.4978	1.5251
	Cycle 5	-.88829*	.25548	.001	-1.4020	-.3746
Cycle 5	No washing	.19001	.25548	.461	-.3237	.7037
	Cycle 1	2.30720*	.25548	.000	1.7935	2.8209
	Cycle 2	2.26037*	.25548	.000	1.7467	2.7740
	Cycle 3	1.89974*	.25548	.000	1.3861	2.4134
	Cycle 4	.88829*	.25548	.001	.3746	1.4020

*. The mean difference is significant at the .05 level.

B5ii- Multiple comparisons for washing cycles (Helmitnh eggs) of spring onions-Bulbs

Dependent Variable: Log Helminth eggs

LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
No washing	Cycle 1	.36308*	.15627	.024	.0489	.6773
	Cycle 2	.36308*	.15627	.024	.0489	.6773
	Cycle 3	-.03207	.15627	.838	-.3463	.2821
	Cycle 4	.19075	.15627	.228	-.1234	.5050
	Cycle 5	.17939	.15627	.257	-.1348	.4936
Cycle 1	No washing	-.36308*	.15627	.024	-.6773	-.0489
	Cycle 2	.00000	.15627	1.000	-.3142	.3142
	Cycle 3	-.39514*	.15627	.015	-.7093	-.0809
	Cycle 4	-.17232	.15627	.276	-.4865	.1419
	Cycle 5	-.18369	.15627	.246	-.4979	.1305
Cycle 2	No washing	-.36308*	.15627	.024	-.6773	-.0489
	Cycle 1	.00000	.15627	1.000	-.3142	.3142
	Cycle 3	-.39514*	.15627	.015	-.7093	-.0809
	Cycle 4	-.17232	.15627	.276	-.4865	.1419
	Cycle 5	-.18369	.15627	.246	-.4979	.1305
Cycle 3	No washing	.03207	.15627	.838	-.2821	.3463
	Cycle 1	.39514*	.15627	.015	.0809	.7093
	Cycle 2	.39514*	.15627	.015	.0809	.7093
	Cycle 4	.22282	.15627	.160	-.0914	.5370
	Cycle 5	.21145	.15627	.182	-.1027	.5257
Cycle 4	No washing	-.19075	.15627	.228	-.5050	.1234
	Cycle 1	.17232	.15627	.276	-.1419	.4865
	Cycle 2	.17232	.15627	.276	-.1419	.4865
	Cycle 3	-.22282	.15627	.160	-.5370	.0914
	Cycle 5	-.01137	.15627	.942	-.3256	.3028
Cycle 5	No washing	-.17939	.15627	.257	-.4936	.1348
	Cycle 1	.18369	.15627	.246	-.1305	.4979
	Cycle 2	.18369	.15627	.246	-.1305	.4979
	Cycle 3	-.21145	.15627	.182	-.5257	.1027
	Cycle 4	.01137	.15627	.942	-.3028	.3256

*. The mean difference is significant at the .05 level.

B6-ANOVA for role of the market environment

		Sum of Squares	df	Mean Square	F	Sig.
Log thermotolerant coliforms	Between Groups	48.630	2	24.315	77.953	.000
	Within Groups	10.293	33	.312		
	Total	58.923	35			
Log Helminths	Between Groups	17.275	2	8.637	7.570	.002
	Within Groups	37.652	33	1.141		
	Total	54.926	35			

B6i- Multiple Comparisons for role of the market environment

LSD

Dependent Variable	(I) Market environment	(J) Market environment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Log thermotolerant coliforms	European market	Ayigya market	-2.06529*	.22801	.000	-2.5292	-1.6014
		Racecourse market	-2.72961*	.22801	.000	-3.1935	-2.2657
	Ayigya market	European market	2.06529*	.22801	.000	1.6014	2.5292
		Racecourse market	-.66432*	.22801	.006	-1.1282	-.2004
	Racecourse market	European market	2.72961*	.22801	.000	2.2657	3.1935
		Ayigya market	.66432*	.22801	.006	.2004	1.1282
Log Helminths	European market	Ayigya market	-1.45167*	.43607	.002	-2.3389	-.5645
		Racecourse market	-1.48667*	.43607	.002	-2.3739	-.5995
	Ayigya market	European market	1.45167*	.43607	.002	.5645	2.3389
		Racecourse market	-.03500	.43607	.937	-.9222	.8522
	Racecourse market	European market	1.48667*	.43607	.002	.5995	2.3739
		Ayigya market	.03500	.43607	.937	-.8522	.9222

*. The mean difference is significant at the .05 level.

APPENDIX C

T-Test results of spring onions before and after assessment of non-treatment interventions at the kitchen

C1- T- test for initial and final load of Thermotolerant coliforms on spring onions (Salt solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	initial load of thermotolerant coliforms - Final load of thermotolerant coliform	-3.58605	1.69044	.29883	-4.19552	-2.97658	-12.000	31	.000

C2- T-test for initial and final load of Helminth eggs on spring onions (Salt solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	initial load of helminth eggs - final load of helminth eggs	1.12594	.59883	.10586	.91004	1.34184	10.636	31	.000

C3- T-test for initial and final load of Thermotolerant coliforms on spring onions (vinegar solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	initial load of thermotolerant coliforms - final load of thermotolerant coliforms	-3.33605	1.90979	.33761	-4.02460	-2.64749	-9.881	31	.000

C4- T-test for initial and final load of Helminth eggs on spring onions (vinegar solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	initial load of helminth eggs - final load of helminth eggs	1.31250	.69270	.12245	1.06275	1.56225	10.718	31	.000

APPENDIX D

T-test results for spring onions before and after tracking from farm to kitchen

D1-T-test for initial and final load of thermotolerant coliforms on spring onions after tracking (salt solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Initial load of thermotolerant coliform - Final load of thermotolerant coliform	-1.50599	3.07705	.51284	-2.54712	-.46487	-2.937	35	.006

D2- T-test for initial and final load of helminth eggs on spring onions after tracking (salt solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Initial load of thermotolerant coliforms - Final load of thermotolerant coliforms	.75231	1.21768	.20295	.34031	1.16432	3.707	35	.001

D3- T-test for initial and final load of thermotolerant coliofrms on spring onions after tracking (vinegar solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Initial load of thermotolerant coliforms - Final load of thermotolerant coliforms	-1.85042	2.86399	.47733	-2.81945	-.88138	-3.877	35	.000

D4-T-test for initial and final load of helminth eggs on spring onions after tracking (vinegar solution)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Initial load of thermotolerant coliforms - Final load of thermotolerant coliforms	.83758	1.29058	.21510	.40091	1.27425	3.894	35	.000

D5-Reduction of thermotolerant coliforms and helminth eggs on wastewater irrigated spring onions as compared to WHO (2006) proposed standards for wastewater use in agriculture.

Protection measure (examples)	Pathogen reduction	Pathogen reduction(TTC)[b]	Pathogen reduction (Helminth eggs)[c]
Two-day cessation of irrigation on farm	0.5-2 per day	0.55 per day	0.3
Storage of spring onions in baskets overnight	N/A	0.42	0.2
Washing 1 kg spring onions bulbs in half bowl of water (8.5litres) and display vertically in basket at market.	N/A	1.47	0.4
Disinfection of spring onions using 100 ml of vinegar: 500 ml of water at kitchen.	2	2.08	1.0
Disinfection of spring onions with salt (7 ppm) solution at kitchen.	2	2.01	0.3
Overall reduction	**6.0**		
Vinegar solution		**4.52**	**1.9**
Salt solution		**4.45**	**1.2**

Pathogen reduction [a]= WHO (2006) proposed reduction range or level of thermotolerant coliforms
Pathogen reduction [b]= Thermotolerant coliforms reduction achieved in the study
Pathogen reduction [c]= Helminth eggs reduction levels achieved in the study, but not proposed in the WHO (2006) guideline.
N/A=Not available in WHO (2006) guideline