

**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND
TECHNOLOGY (KNUST)**

College of Science

Department of Theoretical and Applied Biology

**EFFECTS OF PRE-HOUSEHOLD TREATMENT PROCEDURES AND
DISSIPATION OF CHLORPYRIFOS RESIDUE ON *LACTUCA SATIVA***

By

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BSc. (Hons) Biological Sciences

A Thesis submitted to the Department of Theoretical and Applied Biology,

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in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (Environmental Science)

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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 acknowledgment has been made in the text.

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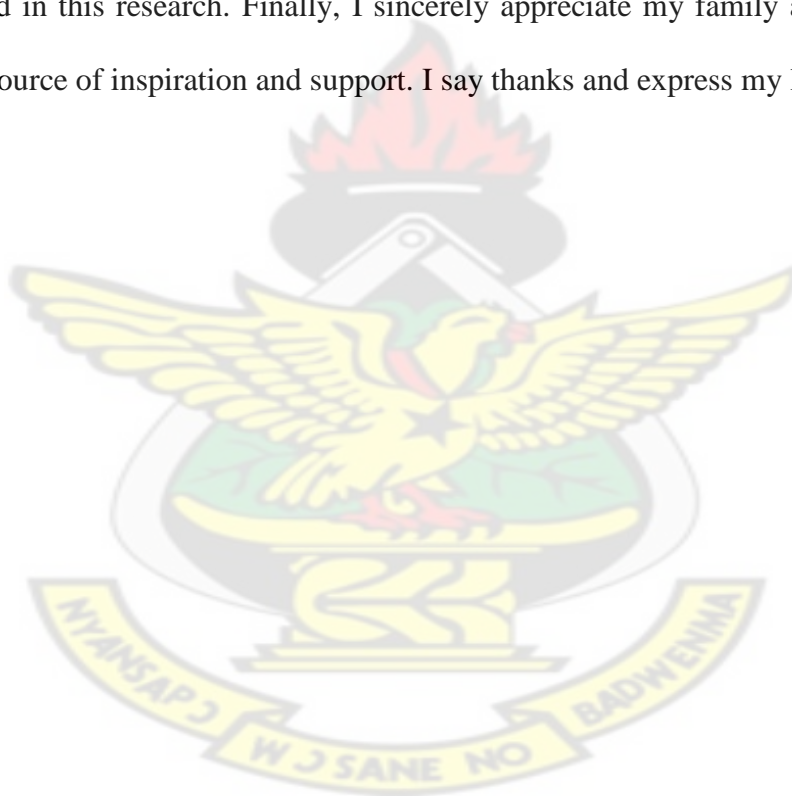
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DEDICATION

This work is dedicated to my mother, Madam Alice Gyimah

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ABSTRACT

This research was organized into two phases. Phase I dealt with the administration of questionnaires to farmers to examine the pesticide use pattern in the study area. Phase II dealt with evaluating the residue levels of chlorpyrifos on *Lactuca sativa* that are likely to have accumulated on *Lactuca sativa* during application and also examining the effect of various household pre-household treatment procedures on reducing the residue levels. Structured questionnaire which was used at the Phase I of this work was to find the knowledge, attitudes and pesticide use practices of farmers. Chlorpyrifos was chosen as the insecticide to be used for Phase II of this work. Chlorpyrifos was then applied at different stages of growth of lettuce. Samples of lettuce were collected at different days after pesticide application [0 (1 hour), 1 day and 7 days] and their residue levels detected using Gas Chromatography. Pre-household treatment procedures such as washing under running tap water, salt water treatment and mild detergent treatment were used to evaluate their effects on chlorpyrifos levels on lettuce. From the questionnaires, it was realised that, the farmers in the study area apply insecticides on their crops to improve the yield and enhance profitability. However, farmers could not state precise application doses to use at specific crop stage and that inappropriate application of pesticides leads to high levels of residues on crops. The results obtained confirm that pesticide residues were indeed present in the lettuce. The highest residue level of 0.059 mg kg⁻¹ was observed on lettuce 0 day (1 hour) after pesticide application. The lowest residue level of 0.002 mg kg⁻¹ was observed on lettuce 7 days after pesticide application. The results also showed that, all the pre-household treatment procedures caused significant reduction in residues levels of chlorpyrifos on the crop. Mild detergent treatment was however more effective compared with the other treatments.

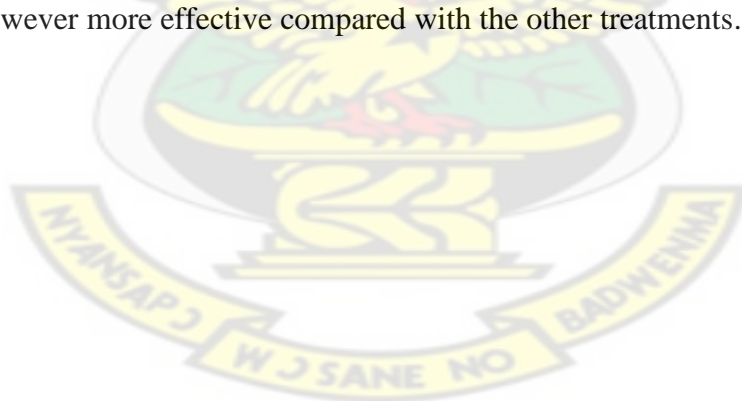


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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Urban food needs in Ghana are growing and as a result, vegetables are grown in urban and peri-urban areas in large quantities to meet this demand. The increase in population and food demand and the susceptibility of vegetables to biotic constraints has resulted in an increase use of pesticides in the production of high-value cash crops and vegetables (Gerken *et al.*, 2001). The consumption of fresh fruits and vegetables is also increasing as consumers strive to eat healthy diets and benefit from the all-year round availability of these products. However, traditional vegetable farming systems (without any chemical input) are incapable of meeting this challenging demand (Gerken *et al.*, 2001).

The use of pesticides in agriculture to maintain plant health and to increase agricultural productivity is increasing at a rapid pace. The importance of pesticides in improving agricultural productivity is evident from the ever increasing use and different varieties of chemicals that are available on the market (Ntow *et al.*, 2006). Pests and diseases which possess big problems in vegetable production require intensive pest management to control

them. Pesticide use is thus a common practice to control pests and diseases in vegetable production in Ghana (Ntow *et al.*, 2006).

Pesticide use in agriculture in the world



dissipated to safer levels. However, the extent and rate of dissipation depends on the nature of pesticide, crop cultural practices and various environmental conditions under which the crop is cultivated (Handa *et al.*, 1999).

Currently organophosphates, carbamates and pyrethroids are the mostly used insecticides in vegetable production in Ghana. Organochlorine pesticides have been banned because of their persistence and bioaccumulation in the environment (Molto *et al.*, 1991). Carbamates and pyrethroids are of limited persistence as compared with organophosphates. Ghanaians however, rely extensively on organophosphates for pest control and vector eradication (Clarke *et al.*, 1997).

Lettuce (*Lactuca sativa*) is an important vegetable cultivated and consumed by both urban and rural dwellers in Ghana. Because of increase in demand by consumers, lettuce is now cultivated all year round. However, pests and diseases are the main biotic factors that militate against the successful cultivation of the crop. Chlorpyrifos [O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate] is a contact organophosphate insecticide intensively used by the farmers in many parts of Ghana as a plant protection measure against pests and diseases of lettuce. Lettuce is subjected to some form of household preparations like washing, dipping in salt solution and removal of non edible parts before actual consumption. Some studies have shown that certain types of post harvest treatments or household preparations may help to reduce pesticide residues (Dhiman *et al.*, 2006; Krol *et al.*, 2000). The effects of these processing techniques on residue levels are extremely important in evaluating the risk associated with ingestion of pesticides residues.

1.2 PROBLEM STATEMENT

Urban agriculture contributes significantly to livelihoods and food security. About 800 million people are engaged in urban and peri-urban agriculture worldwide and contribute about 30% of the world's food supply (UNDP, 1996). In Kumasi, Ghana, more than 12,000 farmers are involved in vegetable farming during dry seasons (Cornish *et al.*, 2001). Urban farmers in Ghana grow about 90% of the main vegetables eaten in the cities (Danso *et al.*, 2003). In several African countries, between 50 and 90% of vegetables consumed are produced within or close to the city (Cofie *et al.*, 2003). The proximity of urban and peri-urban agriculture to consumers ensure freshness of the vegetables and potentially higher nutrients than those stored and transported over long period. This is especially important in Sub-Sahara African where refrigerated transport and cool storage are scarce (Cofie *et al.*, 2003).

However, the increased and inappropriate use of pesticides in vegetable production in Ghana (Obeng-Ofori *et al.*, 2002) has led to an increase of residues on vegetables. Due to lack of education, the farmers do not follow the prescribed dosages and use pesticides at any stage of the crop life without any awareness of the residues and their ill effects on human and environmental health. The high demand for farm produces and low perception of the toxic effects of pesticide residues in food cause farmers not to wait long enough for the residues to break down after spraying before harvesting thus the treated vegetables are picked or harvested without taking into account the pesticide withholding period (Amoah *et al.*, 2006; Bhanti and Taneja, 2007).

Pesticide poses health risks through several exposure pathways including direct occupational exposure, through food or through residues present in the environment. When the residue levels in vegetables exceed the Maximum Residual Levels (MRL's), there are potential health implications (food-borne diseases) to consumers (Ware and Whitacre, 2004). Pesticide residues can remain in the agro-ecosystem and alter metabolism of endemic microorganisms that can lead to radical soil chemistry changes and reduce soil microbial populations resulting in a decrease or changes of soil biological processes. Soil biological processes are very sensitive to chemicals (pesticides) and so the presence of pesticide residues in the soils can result in a decrease or change in the soil biological activity.

1.3 JUSTIFICATION OF STUDY

In Ghana, pesticide residues have been found in fish (Osafo Acquah, 1997), water, sediments, vegetables, human fluids in areas of highly intensive vegetable production areas (Ntow, 2001), dairy products (Darko and Osafo Acquah, 2008) and in soils (Bentum *et al.*, 2006), breast milk (Osei Tutu *et al.*, 2011). The amount of pesticide residues on vegetable crops in Ghana has not been studied extensively. A few isolated studies that observed higher levels of chlorpyrifos on lettuce (Amoah *et al.*, 2006), shallots (Kotey *et al.*, 2008) and tomatoes (Essumang *et al.*, 2008) above the acceptable limits have been carried out. This study therefore seeks to determine the residue levels of chlorpyrifos on lettuce and also gather information on pesticides used pattern by vegetable farmers in Kumasi.

Further, there is no available information on the effects of pre-household treatment procedures in reducing pesticide levels on vegetable in Ghana. The influence of food processing on a specific pesticide-product is important because the behaviour and fate of the

chemical varies with the pesticide as well as with the crop. Therefore, the present investigation is carried out to examine the levels of chlorpyrifos on lettuce, evaluate the impact of various household preparations procedures (fresh water treatment, salt water treatment, detergent treatment) in reducing chlorpyrifos residues levels on lettuce and assess the dissipation of chlorpyrifos.

1.4 GENERAL OBJECTIVE

- The main objective of the study is to measure residue levels of chlorpyrifos on lettuce and examine the effect of pre-household treatment procedures on chlorpyrifos residue on *Lactuca sativa*.

1.5 SPECIFIC OBJECTIVES

- To determine the perception, knowledge and attitudes of farmers in the study area.
- To determine chlorpyrifos residue levels on lettuce.
- To assess the impact of various pre-household treatment procedures in reduction of chlorpyrifos.
- To assess the dissipation of chlorpyrifos at different sampling times (days)

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CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL CHARACTERISTICS OF PESTICIDES

A pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAO, 2002). A pesticide consists of an active ingredient coupled with inert ingredients. The active ingredient kills the

pests, while the inert ingredients facilitate spraying and coating the target plant with the pesticide. They can also contribute other advantages that are not conferred by the active ingredient alone.

How effective the pesticides are at killing the target organisms (efficacy) depends on the properties of the pesticide and the soil, formulation, application technique, agricultural management, characteristics of the crop, environmental or weather conditions, and the nature and behaviour of the target organism (Koehler and Belmont, 1998).

2.2 CLASSIFICATION OF PESTICIDES

Pesticides can be classified in various ways depending on one's interest. Some of the common ways of classifying pesticides are by the target organism it acts on, its mode of action and its chemical nature.

2.2.1 CLASSIFICATION BY TARGET ORGANISM

Pesticides can be classified by target organism (American Medical Association, 1997). For example, Algicides or algaecides is used for the control of algae, avicides for the control of birds, bactericides for the control of bacteria, fungicides for the control of fungi and oomycetes, herbicides for the control of weeds, insecticides for the control of insects, miticides or acaricides for the control of mites, molluscicides for the control of slugs and

snails, nematicides for the control of nematodes, rodenticides for the control of rodents, virucides for the control of viruses (Koehler and Belmont, 1998).

2.2.2 CLASSIFICATION BY CHEMICAL NATURE

One of the most common means of classifying a pesticide is on the basis of similarities in chemical structure. Based on this mode of classification, there are 3 classes of pesticides commonly used in the structural pest control industry. These are the inorganic, botanical (natural) and synthetic organic insecticides (Koehler and Belmont, 1998).

2.2.2.1 Inorganic Pesticides

Inorganic pesticides are those made from compounds that do not contain carbon. Inorganic pesticides are typically derived from minerals or chemical compounds that occur as deposits in nature. Most of these compounds are quite stable and tend to accumulate in the environment. Some act as stomach poisons (borates and boric acid). Others are considered sorptive dusts (silica aerogel, diatomaceous earth) that absorb the waxy layer from the cuticle of pests. Many of the inorganic pesticides are relatively expensive and are only moderately effective in controlling insects and other pests. Common inorganic pesticides are silica aerogel, boric acid, borates, diatomaceous earth, cryolite, copper, and sulphur (Koehler and Belmont, 1998).

2.2.2.2 Botanicals

The botanical pesticides are extracted from various parts (stems, seeds, roots and flower heads) of different plant species. Botanical pesticides usually have a short residual activity

and do not accumulate in the environment or in fatty tissues of warm blooded animals. Many botanical pesticides act as stomach poisons, although pyrethrins act mainly as a contact poison. Common examples of botanical pesticides are pyrethrins, sabidilla, rotenone, nicotine, ryania, neem, and limonene (Koehler and Belmont, 1998).

2.2.2.3 Synthetic Organic Pesticides

Synthetic organic insecticides do not naturally occur in the environment, but are synthesized by man. Since all these compounds have carbon and hydrogen atoms as the basis of their molecules, they are referred to as organic compounds. The four basic types of synthetic organic insecticides are the chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids (Koehler and Belmont, 1998).

2.2.3 CLASSIFICATION BY MODE OF ACTION

Mode of action refers to the mechanism by which the pesticide kills or interacts with the target organism. Pesticides enter the pest body by three common ways; by contact, as stomach poisons or as fumigants. Many pesticides may enter the body by more than one of these possible routes (Koehler and Belmont, 1998).

2.2.3.1 Contact Pesticides

Pesticides in this class kill pests by contacting and entering their bodies either directly through the pest integument (skin) into the blood or by entering the respiratory system through the spiracles. These materials may be applied directly to the pest's body or as a residue on plant or animal surfaces, habitations or other places frequented by the pest. In

cases where residues are used, pests usually contact the pesticide through their feet. Coarse sprays or dusts are a more effective means of applying contact insecticides than are mists or fogs. Most of the synthetic organic compounds act as contact insecticide, although many also confer stomach and fumigant activity (Koehler and Belmont, 1998).

2.2.3.2 Systemic or Stomach Poisons

Systemic pesticides act mainly as stomach poisons. These chemicals are typically applied to one area of a plant or animal and are translocated to another area. Stomach poisons must be swallowed in order to cause death. They may be formulated as liquids, dusts, pastes, granules or baits. In the case of liquids and dusts, the pesticide is usually applied to some substance on which the animal feeds or walks through. Pastes, bait and granules may be formulated with a feeding attractant which is consumed by the pest. Inorganic and botanical (natural) pesticides in general are predominantly stomach poisons. Some synthetic organic pesticides may also act in this capacity (Koehler and Belmont, 1998).

2.2.3.3 Fumigant Pesticides

Fumigants are gaseous poisons which kill pests when they are inhaled or absorbed. Their applications are usually limited to materials, structures or organisms that can be or are enclosed in a tight enclosure. There are many fumigants; some are distinctly odourless, while others are used in conjunction with odourless fumigants as a warning agent because of their odour. Fumigants leave no residue on the food after it has been used and therefore can be used safely on food products. Some combine with commodities to produce corrosive or undesirable gases. When properly used, a fumigant is non-flammable and unlike any other forms of pesticides, kills all the developmental stages of an animal. In the case of insects, this includes the egg, larvae, pupae, and adults. When a fumigant reaches the appropriate concentration, it will kill pests quicker than any other pesticide (Koehler and Belmont, 1998).

2.2.3.4 Insect Growth Regulators

Insect growth regulators are chemicals that affect the ability of insects to grow and mature normally. They often mimic the growth hormones that occur naturally within the insect's body. Because mammals do not molt like insects do, most insect growth regulators are not very toxic to man and domestic animals. Common insect growth regulators are methoprene (Precor), hydroprene (Gentrol, Gencor), fenoxycarb (Torus), and hexaflumuron (Sentricon) (Koehler and Belmont, 1998).

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2.3 EFFECTS OF PESTICIDE USE ON HUMAN HEALTH

If the credits of pesticide use include enhanced economic potential in terms of increasing production of food and fibre, and amelioration of vector-borne diseases, then their debits have resulted in serious health implications to man and the environment. There is now overwhelming evidence that some of these chemicals do pose a potential health risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993). No segment of the population is completely protected against exposure to pesticides and their potentially serious health effects though the people of developing countries are at a higher risk (WHO, 1990).

To analyze the possible side effects of pesticide use on human health, a distinction has to be made between occupational health hazards and pesticide residues in food products and drinking water. The World Health Organization and the UN Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die (Miller, 2004). The world-wide deaths and chronic diseases due to pesticide poisoning number about 1 million per year. The

high risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers (Environews Forum, 1999).

Clarke *et al.*, 1997 undertook a field study to examine the extent of pesticide-associated symptoms in farmers involved in irrigation projects in Ghana. About 36% of the interviewed farmers had experienced negative side effects after applying pesticides. The most significant symptoms included headache, dizziness, fever, blurred vision, and nausea/vomiting. Additionally, studies have indicated that pesticide exposure is associated with long-term health problems such as respiratory problems, memory disorders, dermatologic conditions (Arcury *et al.*, 2003), cancer, depression, neurological deficits, miscarriages, and birth defects (Stallones and Beseler, 2002). The symptoms observed by Clarke *et al.*, 1997 were more prevalent with the farmers than with a control group of teachers in the same region. Blood tests for cholinesterase as an indication of residues of organophosphates showed a lower activity band in the farmers compared to the teachers. Cholinesterase levels were influenced by the duration and frequency of pesticide handling.

A long-term study on possible poisoning caused by pesticides was carried out by Adetola *et al.*, 1999. The research analyzed organs of the body, body fluids, foods and drinks. Out of the 1,215 toxicological cases that were analyzed, 963 cases were tested positively for chemical poisoning. 30% of cases of chemical poisoning were directly related to the misuse of pesticides. The main causes for deaths were carbamates (126 cases), organophosphorous pesticides (66 cases) and organochlorines (74 cases).

Occupational exposure of farmers to various pesticide products influences the health status of the farmers. From the work of Ntow *et al.*, 2007, it revealed that high risks practices such as

lack of personal protective clothing, short re-entry intervals, and wrong direction of spraying of pesticides by hand or knapsack sprayer had an impact on the health status of the farmers. The decreases in cholinesterase (ChE) activity were seen in self-reported symptoms attributable to pesticide exposure. About 97% of the population of vegetable farmers at Akumadan (exposed group) had reported symptoms attributable to pesticide exposure in the week preceding the survey with the frequent symptoms being body weakness and headache. The lack of protective measures such as the standard protective clothing's is a problem not only at the farm level, but also during transportation, distribution and disposal of waste. Empty containers are often re-used for household water or food items. Also, in case of accidents, no first aid kits and showers are available, which lead to more serious consequences for the victim (EPA, 1997).

2.4 FATE OF PESTICIDES IN THE ENVIRONMENT

A pesticide's fate is described by how and where it enters the environment, how long it lasts and where it goes. How a pesticide enters the environment is the first step in determining its fate. Initial distribution is determined by the method of application, the amount, timing, frequency and placement. Weather conditions during application can also affect initial distribution. Land form (topography), vegetation type and density, soil conditions, and the proximity of water bodies also are important (EXTONET, 1998).

After application of pesticide, the pesticide may break down, be redistributed within the application site or move off site. The amount of pesticide that migrates from the intended application area is influenced by the particular chemical's properties: its propensity for binding to soil, its vapor pressure, its water solubility, and its resistance to degradation over time (Kellogg, 2000). Soil factors such as its texture, its ability to retain water, and the

amount of organic matter contained in it, also affect the amount of pesticide that will leave the area. Off site movement includes movement to groundwater, surface water, and the atmosphere. It also includes removal of crops contaminated with pesticide from the site. Break down and movement occur simultaneously. In many cases, the two processes together determine pesticide dissipation at the point of measurement. The rate at which pesticides breakdown depends on their reactivity in each media (air, soil, water, plants, animals). Each pesticide has unique properties that determine reactivity. Some pesticides are sensitive to acidic and/or basic conditions (pH), others are sensitive to sunlight, microbial attack, or plant and animal metabolism (Kellogg, 2000).

How long a pesticide lasts in the environment is determined by a number of factors including how much is introduced and how it is distributed; its reactivity in the environmental media, and the conditions of the media. Pesticide persistence is often expressed in terms of half-life. This is the time required for one-half the original quantity to break down. Pesticides can be divided into three (3) categories based on half-lives: non persistent (less than 30 days), moderately persistent (30 to 100 days) and persistent (greater than 100 days). Because half-life values can vary considerably depending on environmental conditions, they are often reported as a range for each media (EXTONET, 1998).

Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments, and food (Miller, 2004). Pesticides affect the environment by point-source pollution and nonpoint-source pollution. The point-source is the contamination that comes from a specific and identifiable place; including pesticide spills, wash water from cleanup sites, leaks from storage sites, and improper disposal of pesticides

and their containers. The nonpoint source is the contamination that comes from a wide area, including the drift of pesticides through the air, pesticide runoff into waterways, pesticide movement into ground water (Toth and Buhler, 2009). Environmentally-sensitive areas to the pesticides are: where ground water is near surface ; heavily populated with people; populated with livestock and pets; near the habitats of endangered species and other wildlife; near honey bees; near food crops and ornamental plants (Toth and Buhler, 2009). Sensitive plants and animals as well as the water quality of water bodies in field margins can be affected either directly or indirectly (Cessna *et al.*, 2005).

Factors which influence plant uptake and metabolism of pesticides are physicochemical behaviour, formulation, droplet size and application technique, precipitation or rainfall and relative humidity, temperature, sunlight, plant species and physiological differences, e.g. stomata, upper or lower leaf surface, hairs, waxes, and time of application during the vegetative period. Similarly factors which influence root uptake and degradation of pesticides in soil are physicochemical behaviour, application method and amount, physicochemical and biochemical reactions in the soil, climatic factors and plant development (Führ 1991). The degree of plant uptake is determined partially by the pesticide's water solubility. Plant uptake of pesticides prevents runoff or leaching (Kerle *et al.*, 2007).

Pesticides may volatilize or be blown away by the wind. Volatilization is the process of solids or liquids converting into a gas, which can move away from the initial application site. This movement is called vapour drift. Various factors are responsible for the pesticide volatilization from foliage. Pesticides on foliage are most susceptible to volatilization after application, because over time, pesticides become incorporated into surface waxes. The

dominant factors that influence the volatilization of pesticides from crops are the physicochemical properties of the pesticide, the persistence on the plant surface and the environmental conditions (wind, temperature, and air humidity). The primary effect of wind on pesticide disappearance from foliage is through turbulent transfer of volatilized pesticide from plant surfaces to the atmosphere (Spencer *et al.*, 1973). Temperature affects pesticide disappearance from foliage through its influence on pesticide vapour pressure and volatility (Harper *et al.*, 1983). The higher the temperature, the greater the volatilization rate. Pesticides with low vapour pressure index values have a low potential to volatilize while those with high vapour pressure index values have a high potential to volatilize (Kerle *et al.*, 2007). Sunlight affects pesticide disappearance through photochemical alteration of the pesticide. High relative humidity has been reported to both increase pesticide persistence on plants by facilitating foliar absorption and decrease persistence by favouring volatilization (Willis and McDowell, 1987).

2.5 ROUTES OF EXPOSURE TO PESTICIDES

A pesticide can enter the body through three routes of exposure; inhalation, ingestion and skin penetration or dermal exposure (Jaga and Dharmani, 2003; US EPA, 2007). Inhalation exposure can happen if you breathe air containing pesticide as a vapour, as an aerosol, or on small particles like dust. Ingestion or Oral exposure happens when you eat food or drink water containing pesticides. Dermal exposure happens when your skin is exposed to pesticides. Depending on the situation, pesticides could enter the body by any one or all of these routes (US EPA, 2007).

Direct contact with the pesticide is the most prevalent way that pesticides penetrate the skin, but exposure through the skin may also occur as a result of contact with pesticides in air and

water (Arcury *et al.*, 2003). Workers can be exposed to pesticides in their workplaces as a result of their occupation. Irrespective of whether the job involves pesticide use, the presence of the chemical in the work environment constitutes direct occupational exposure.

Workers who handle pesticides directly are at higher risk of exposure than workers who do not handle pesticides directly. Most occupational exposures to pesticides are through inhalation or dermal exposure, and in some instances by ocular exposure (Sullivan and Blose 1992., Schenker *et al.*, 1992). Similarly, pesticide use can involve more than one route of exposure if precautions are not taken. A pesticide which is sprayed can be inhaled during use; penetrate through the skin during mixing and application; and be ingested through food if not washed off from hands or food before eating (US EPA, 2007).

2.6 SOURCES OF PESTICIDE EXPOSURE

Various sources of pesticide exposure to humans include (US EPA, 2007):

- Food: Most of the foods we eat have been grown with the use of pesticides. Therefore, pesticide residues may be present inside or on the surfaces of these foods.
- Home and Personal Use Pesticides: Pesticides are used in and around the home to control insects, weeds, mold, mildew, bacteria, lawn and garden pests and to protect pets from pests such as fleas. Pesticides may also be used as insect repellants which are directly applied to the skin or clothing.
- Pesticides in Drinking Water: Some pesticides that are applied to farmland or other land structures can make their way in small amounts to the ground water or surface water systems that feed drinking water supplies.

- Occupational Exposure to Pesticides: Pesticide applicators, vegetable and fruit pickers and others who work around pesticides can be exposed due to the nature of their jobs.

2.7 PESTICIDE DEGRADATION

Pesticide degradation is the process of pesticide breakdown after application. It is the major process of loss for most pesticides after their application (Frere, 1975). Pesticides are broken down by microbes, chemical reactions, and light or photodegradation. The rate of pesticides degradation depends on environmental conditions and the chemical characteristics of the pesticide. Pesticides that break down quickly generally do not persist in the environment or on the crop. However pesticides that break down too rapidly may only provide short-term control. Pesticide degradation or break down is usually beneficial because it change most of the pesticide residue in the environment into nontoxic or harmless compounds. Degradation can be detrimental when a pesticide is destroyed before the target pest has been controlled (Waldron, 1992).

2.7.1 Microbial Degradation of Pesticide

This is the breakdown of pesticides by microorganisms that use pesticides as their source of food. Most microbial degradation of pesticides occurs in the soil. The rate of microbial degradation is affected by soil conditions such as moisture, temperature, aeration, pH and organic matter content because of their direct influence on microbial growth and activity. The rate of pesticide application is also a factor that influences microbial degradation. Rapid microbial degradation is more likely when the same pesticide is used repeatedly in a field. Repeated applications actually stimulate the build-up of organisms that are effective in

degrading the chemical. As the population of these organisms increases, degradation accelerate and the amount of pesticide available to control the pest is reduced. The possibility of very rapid pesticide breakdown is reduced by using pesticides only when necessary and by avoiding repeated applications of the same chemical (Waldron, 1992).

2.7.2 Chemical Degradation of Pesticide

This is the breakdown of pesticides by chemical reactions in the soil. Temperature, moisture, pH and adsorption in addition to chemical and physical properties of the pesticide, determine which chemical reactions takes place and how quickly they occur. Hydrolysis, a breakdown process in which the pesticide reacts with water is one of the common degradation reactions (Waldron, 1992).

2.7.3 Photodegradation of Pesticide

Photochemical degradation (photolysis) is the degradation process whereby radiant energy in the form of photons breaks the chemical bonds of a molecule. That is the breakdown of pesticides by light, particularly sunlight. Photodegradation can destroy pesticides on foliage, on the surface of the soil and even in the air. Pesticides applied to foliage are more exposed to sunlight than pesticides that are incorporated into the soil. All pesticides are susceptible to photodegradation to some extent. The rate of breakdown is influenced by the intensity and spectrum of sunlight, length of exposure, and the properties of the pesticide (Waldron, 1992).

2.8 PHYSICAL AND CHEMICAL PROPERTIES OF CHLORPYRIFOS

Chlorpyrifos is an organophosphate insecticide and is among the most frequently used pesticides to control pests and diseases of lettuce in Ghana. This insecticide was the one selected for this study. Chlorpyrifos [O,O-Diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum and non-systemic insecticide. It is used on a wide range of crops including vegetables, citrus fruits, peaches, cereals and tobacco. It has a molecular weight of 350.6g and an empirical formula $C_9H_{11}Cl_3NO_3PS$. Its chemical structure is shown in figure 2.1.

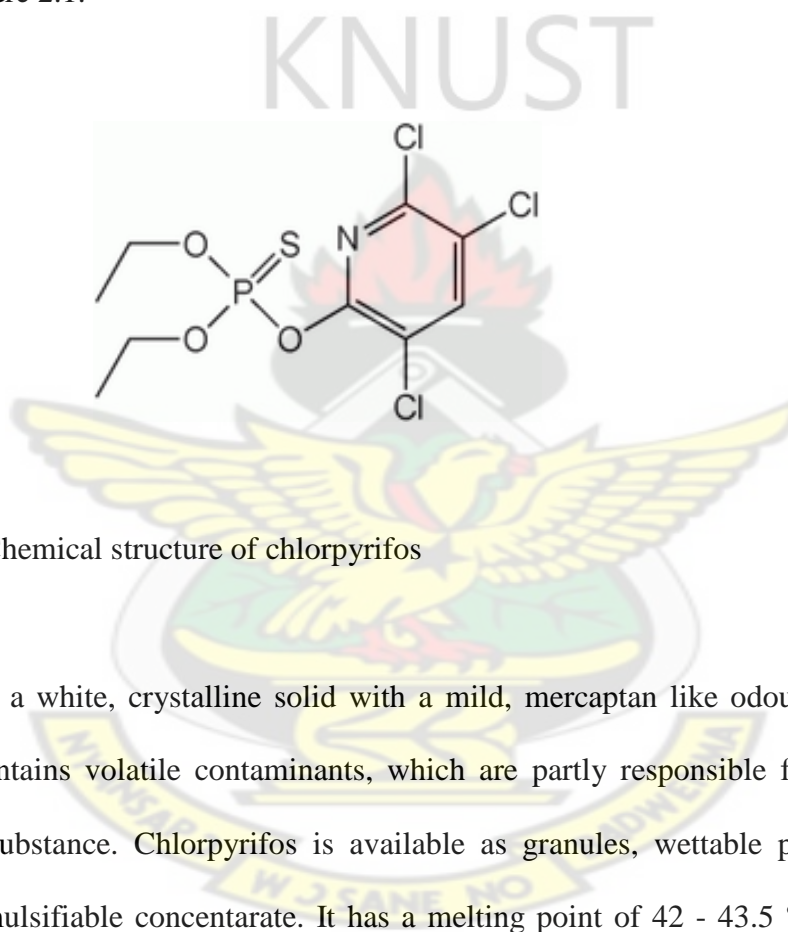


Figure 2.1: Chemical structure of chlorpyrifos

Chlorpyrifos is a white, crystalline solid with a mild, mercaptan like odour. The technical grade often contains volatile contaminants, which are partly responsible for the offensive odour of the substance. Chlorpyrifos is available as granules, wettable powder, dustable powder and emulsifiable concentrate. It has a melting point of 42 - 43.5 °C and a vapour pressure of 2.4×10^{-5} mm Hg (at 25 °C). Chlorpyrifos is not readily soluble in water but soluble in acetone, benzene, chloroform, methanol and iso-octane (Kidd and James, 1991).

When chlorpyrifos is exposed to UV light or to sunlight, it undergoes hydrolysis in the presence of water to liberate 3,5,6-trichloro-2-pyridinol, which undergoes further

decomposition to diols and triols and ultimately cleavage of the ring to fragmentary products (Smith, 1968). Hydrolysis of chlorpyrifos in water occurs least readily at about pH 6 and very readily above pH 8 (Smith, 1968). The chemical process involved in the hydrolysis is presented in figure 2.2.



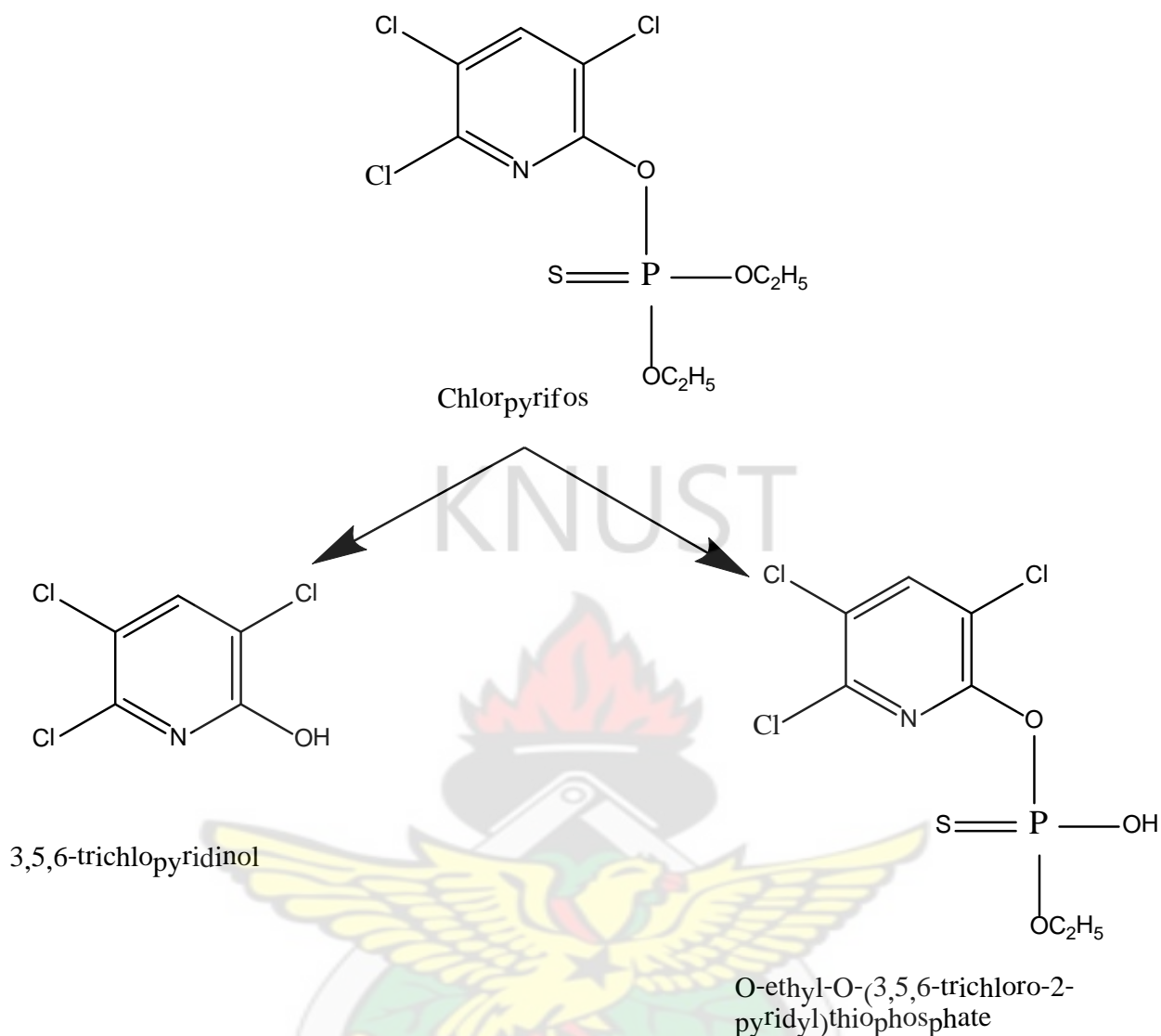


Figure 2.2: Chemical degradation pathway of chlorpyrifos

2.8.1 MODE OF ACTION OF CHLORPYRIFOS

Chlorpyrifos acts as a contact poison by affecting the normal function of the nervous system (US EPA, 1999). Chlorpyrifos affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter (Smegal, 2000). When insects are exposed to chlorpyrifos, the compound binds to the active site of the cholinesterase (ChE) enzyme, which prevents breakdown of ACh in the synaptic cleft. The resulting accumulation of ACh

in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death (Karanth and Pope 2000). There is no evidence that chlorpyrifos is mutagenic, teratogenic or carcinogenic.

2.8.2 FATE OF CHLORPYRIFOS IN THE ENVIRONMENT

Chlorpyrifos enters the environment through direct application to crops, lawns, domesticated animals etc. Chlorpyrifos may also enter the environment through volatilization, spills, and the disposal of chlorpyrifos waste. Chlorpyrifos that has been applied to the soil generally stays in the area where it has been applied because it sticks tightly to soil particles. Because of this, there is a low chance that chlorpyrifos will be washed off the soil and enter local water systems. Also, chlorpyrifos does not mix well with water and if it gets into the natural waters, it will be in small amounts and will remain on or near the surface and evaporate. Volatilization is the major way in which chlorpyrifos disperses after it has been applied. Once in the environment (soil, air, or water), chlorpyrifos is broken down by sunlight, bacteria, or other chemical processes. Chlorpyrifos undergoes hydrolysis in the environment to yield 3,5,6-trichloro-2-pyridinol (TCP) as the major degradation product. (US EPA, 2002).

2.8.2.1 Plants

Chlorpyrifos is not expected to be taken up from soil by the roots of plants (Tomlin, 2006). Though some chlorpyrifos may be taken up by plants through leaf surfaces, much of the applied chlorpyrifos is usually lost by volatilization, and very little is translocated throughout the plant (Roberts and Hutson, 1999). Chlorpyrifos taken up by plant tissues is primarily metabolized to 3,5,6-trichloro-2-pyridinol (TCP), which is then stored as glycoside conjugates (Roberts and Hutson, 1999; Tomlin, 2006). Studies report chlorpyrifos residues

remain on plant surfaces for 10 to 14 days after application. Although most of the chlorpyrifos applied to plants is lost through volatilization or converted to TCP and sequestered, desulfuration to chlorpyrifos oxon on plant surfaces has been reported (Roberts and Hutson, 1999). Dislodgeable foliar residues of chlorpyrifos comprise a rather small proportion of the total residue present and decline even more rapidly than total residues. Dislodgeable residues typically represent less than 10 percent of total residues, and half-lives of 0.5 to 3 days are common. Chlorpyrifos may be toxic to some plants, such as lettuce (McEwen and Stephenson, 1979). Data indicate that this insecticide and its soil metabolites can accumulate in certain crops (U.S. Public Health Service, 1995).

2.8.2.2 Soil

Chlorpyrifos is moderately persistent in soils. Chlorpyrifos is stable in soils with reported half-lives ranging between 7 and 120 days. Studies have found chlorpyrifos in soils for over one year following application. Soil persistence may depend on the formulation, rate of application, soil type, climate and other conditions. (Kamrin, 1997; Roberts and Hutson, 1999). Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis, dechlorination, and soil microbes (Kamrin, 1997; Roberts and Hutson, 1999). Chlorpyrifos binds strongly to soils, is relatively immobile, and has low water solubility.

In contrast, its degraded TCP adsorbs weakly to soil particles and is moderately mobile and persistent in soils (Kamrin, 1997; US EPA, 1999). The major degradates of chlorpyrifos found in soils are similar to the metabolites created by plants and animals. The degradates are formed by oxidative dealkylation or hydrolysis to diethyl phosphates and TCP (Roberts and Hutson, 1999). The soil half-life of chlorpyrifos was from 11 to 141 days in seven aerobic soils ranging in texture from loamy sand to clay and with soil pHs from 5.4 to 7.4.

Chlorpyrifos is less persistent in soils with a higher pH (Kamrin, 1997; US EPA, 1999), Soil half-life is not affected by soil texture or organic matter content. In anaerobic soils, the half-life is 15 days in loam and 58 days in clay soil. When applied to moist soils, the volatility half-life of chlorpyrifos was 45 to 163 hours, with 62% to 89% of the applied chlorpyrifos remaining on the soil after 36 hours (Racke, 1992).

2.8.3 FATE OF CHLORPYRIFOS IN HUMANS

Chlorpyrifos moves to all parts of the body after exposure. Organophosphate insecticides share a common mechanism of toxicity, through inhibitory effects on cholinesterase enzymes in the nervous system (Reigart and Roberts, 1999). One chemical reaction that occurs in humans is the transformation at the double bond of the central phosphorus atom from sulphur to oxygen. This metabolic reaction takes place in the liver and results in activation of the organophosphate to a more potent inhibitor of cholinesterase enzymes (Reigart and Roberts, 1999).

Thus, the chlorpyrifos is not toxic to the human body but when the body tries to break it down, it creates a toxic form. This toxic form, called chlorpyrifos oxon (fig: 2.3), binds permanently to enzymes which control the messages that travel between nerve cells. When chlorpyrifos binds to, too many of the enzymes, nerves and muscles do not function correctly. The body then must make more enzymes so that normal nerve function can resume. The body can break down and excrete most of the unbound chlorpyrifos in faeces and urine within a few days. Chlorpyrifos that finds its way into the nervous system may stay there much longer (NPIC document, 2010).

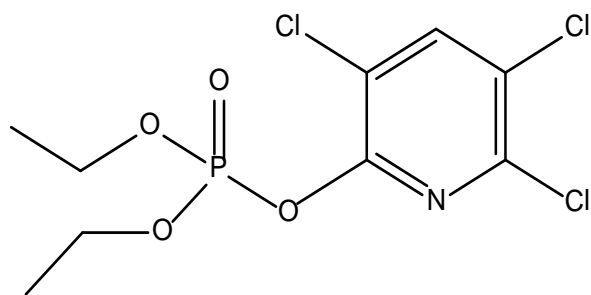


Figure 2.3: Chemical structure of chlorpyrifos oxon

2.8.4 TOXICITY SYMPTOMS IN HUMANS

Chlorpyrifos is moderately toxic to humans. Chlorpyrifos can be absorbed through all routes of exposure. Signs and symptoms typically develop within minutes to hours after an acute exposure to chlorpyrifos. Acute exposure depends on the route and extent of exposure. Inhalation may cause respiratory and ocular effects, often within a few minutes of exposure. When toxic amounts are inhaled, the first effects are usually respiratory and may include bloody or runny nose, coughing, chest discomfort, difficult or short breath, and wheezing due to constriction or excess fluid in the bronchial tubes. Plasma cholinesterase levels activity has been shown to be inhibited when chlorpyrifos particles are inhaled. Inhalation of chlorpyrifos may cause absorption of the insecticide through the mucous membranes, resulting in systemic intoxication (OHS, 1986). Ingestion may cause vomiting, diarrhoea and skin absorption may result in localized sweating and muscle tremors in the area where pesticide absorption took place. Eye contact may cause pain, bleeding, tears, pupil constriction, and blurred vision (Reigart and Roberts, 1999).

In general, initial signs and symptoms include tearing of the eyes, runny nose, increased saliva and sweat production, nausea, dizziness and headache. Signs of progression include muscle twitching, weakness or tremors, lack of coordination, vomiting, abdominal cramps, diarrhoea, and pupil constriction with blurred or darkened vision (Reigart and Roberts, 1999; Thompson and Richardson, 2004). Signs of severe toxicity include increased heart rate, unconsciousness, loss of control of the urine or bowels, convulsions, respiratory depression, and paralysis (Reigart and Roberts, 1999; Thompson and Richardson, 2004). Children may experience different signs and symptoms from exposure to chlorpyrifos than adults, and diagnosis of poisoning in general may be more difficult. Commonly reported signs and symptoms in poisonings with children include seizures, flaccid muscle weakness, pupil constriction, excess salivation and mental status changes including lethargy and coma. Some of the typical symptoms seen in adults, such as decreased heart rate, muscle twitching, increased tear production, and sweating, are less common in children (Reigart and Roberts, 1999).

2.9 PESTICIDE RESIDUES

Pesticide residues are the small amounts of pesticides that can remain in or on food and in the environment. The idea to regulate pesticide residues to safe levels was originally introduced by the Joint FAO/WHO Expert Committee on Food Additives in 1995. The Codex Committee on Pesticide Residues (CCPR) is a subsidiary body of the Codex Alimentarius Commission that advises on all matters related to pesticide residues. Its primary objective is to develop Maximum Residue Levels in order to protect the health of the consumer while facilitating international trade.

The Maximum Residual Levels (MRL's) set by Codex Committee on Pesticide Residues are regulatory guidelines and the basis for comparison to determine whether the pesticide residue concentrations in the agricultural crops are within acceptable limits in relation to human and environmental health. That is it checks whether the maximum concentration for a pesticide residue in a specific agricultural crop is in accordance with Good Agricultural Practices (GAPs). Good Agricultural Practices are a collection of principles to apply for on-farm production and post-production processes, resulting in safe and healthy [food](#) and non-food agricultural products, while taking into account economical, social and environmental [sustainability](#). GAPs are those procedures designed to enhance the safety of vegetables by the implementation of safer harvesting, handling, production and packing practices. It therefore minimizes the contamination of fresh produce with pesticides and microbial pathogens in every step from production to food preparation. The goal is to prevent the contamination of fresh produce either in the natural environment or in the handling, packing, and selling of produce (FAO/WHO, 2003).

Pesticide residues above the tolerance levels (MRL) in the crop at harvest are a cause of great concern globally and nationally. Whenever pesticides are applied, their residues remain on treated surfaces for some time. The chemical properties, frequency of application and environmental factors determine how much residue will be present. Residues are important in certain circumstances and necessary for some types of pest control where their presence provides continuous control. Pesticides that move off-site or miss the intended application site can also remain as residues in soil, water, or on surfaces (P.I.O, 2005).

There are three potential sources of pesticide residues in food grains and in the soil:

- application of pesticides to protect the growing crop;
- contamination of the environment by highly stable pesticides previously applied for other purposes; and
- application of insecticides to protect the harvested crop during storage and handling.

2.9.1 PESTICIDE RESIDUES ON VEGETABLES

Pesticide residues in crops are a direct result of the application of pesticides to crops growing in the fields, and to a lesser extent to pesticide residues remaining in the soil (Businelli *et al.*, 1992).

In Ghana, pesticide residue levels observed on lettuce exceeded the Maximum Residue Limit (MRL). More than 60% of the lettuce samples analyzed had two or more types of pesticide residues. Chlorpyrifos was detected on 78% of the lettuce, lindane on 31%, endosulfan on 36%, lambda cyhalothrin on 11%, and DDT on 36%. Chlorpyrifos was the only pesticide with higher residue levels in Kumasi. Most of the residues measured exceeded the maximum residue limit for consumption (Amoah *et al.*, 2006).

A survey of sixty farmers from the Volta region of Ghana revealed inappropriate pesticide application practices. Residue analysis showed the presence of chlorpyrifos, DDT,

cypermethrin, and dimethoate in shallots. The levels of chlorpyrifos exceeded the Codex maximum residue level in most samples, whilst residue levels of all the other pesticides (DDT, cypermethrin, and dimethoate) detected in samples were lower than Codex MRLs. More than 50% of samples had chlorpyrifos levels above Codex MRLs (Kotey *et al.*, 2008). A study by Essumang *et al.*, 2008 evaluated the residue levels of select pesticides used on tomato crops in Ghana. It was to ascertain the pesticides that are likely to have accumulated in the tomatoes during application. The results obtained confirm that pesticide residues were indeed present in the tomatoes. Analysis of organochlorine and organophosphorus residue levels in the fruits indicated that chlorpyrifos, which is an active ingredient of pesticides registered in Ghana under the trade name Dursban 4E or Terminus 480 EC for use on vegetables, has the greatest residue level of 10.76 mg kg^{-1} . The lowest residue level observed was that of pirimiphos-methyl with 0.03 mg kg^{-1} .

From the work conducted by Darko and Akoto (2008), ethyl-chlorpyrifos was observed at an average level of $0.211 \pm 0.010 \text{ mg kg}^{-1}$ in 42% of tomato, $0.096 \pm 0.035 \text{ mg kg}^{-1}$ in 10% of eggplant and $0.021 \pm 0.013 \text{ mg kg}^{-1}$ in 16% of pepper was below the 0.5 mg kg^{-1} MRL. Dichlorvos was the most frequently detected residue in all the samples analyzed. Levels of malathion in tomatoes ($0.120 \pm 0.101 \text{ mg kg}^{-1}$) and pepper ($0.143 \pm 0.042 \text{ mg kg}^{-1}$) exceeded the MRL of 0.1 mg kg^{-1} .

The European Union established a Monitoring of Pesticide Residues in products of plant origin program in 1996 to check the levels of pesticide residues on vegetables. In 1996, seven pesticides (methamidophos, thiabendazole, maneb, acephate, chlorpyrifos, endosulfan, and benomyl group) were analyzed in apples, tomatoes, lettuce, strawberries and grapes. For each pesticide 5.2% of the samples were found to contain residues and 31% of that had residues

higher than the respective MRL for that specific pesticide. Lettuce was the crop with the highest number of positive results, with residue levels exceeding the MRLs more frequently than in any of the other crops investigated (EC, 2001).

In 1997, 13 pesticides were assessed in five commodities (mandarins, pears, bananas, beans, and potatoes). Residues of chlorpyrifos exceeded MRLs in 24% of the samples, followed by methamidophos (18%), and iprodione (13%). With regard to the commodities investigated, 34% contained pesticide residues below the MRL, and 1% contained residues at levels above the MRL. In mandarins, pesticide residues were most frequently found at levels below the MRL (69%), followed by bananas (51%), pears (28%), beans (21%) and potatoes (9%). MRLs were exceeded most often in beans (1.9%), followed by mandarins (1.8%), pears (1.3%), and bananas and potatoes (0.5%) (E.C, 2001).

In 1998, four commodities (oranges, peaches, carrots, spinach) were analyzed for 20 pesticides. About 32% contained residues of pesticides below MRL, and 2% above the MRL. Residues at or below the MRL were found most often in oranges (67%), followed by peaches (21%), carrots (11%) and spinach (5%). MRL values were exceeded most often in spinach (7.3%), followed by peaches (1.6%), carrots (1.2%) and oranges (0.7%) (E.C, 2001).

In another study, four commodities (cauliflower, peppers, wheat grains, and melon) were analyzed. Residues of methamidophos exceeded MRLs most often (8.7%), followed by the maneb group (1.1%), thiabendazole (0.57%), acephate (0.41%) and the benomyl group (0.35%). The MRL for methamidophos was exceeded most often in peppers and melons (18.7 and 3.7%, respectively). The residues of the maneb group exceeded the MRL most often in cauliflower (3.9%); residues of thiabendazole exceeded the MRL most often in melons (2.8%

of the melon samples). With regard to all the commodities investigated, around 22% of samples contained residues of pesticides at or below the MRL and 8.7% above the MRL. Residues at or below MRL were found most often in melons (32%), followed by peppers (24%), wheat grains (21%) and cauliflower (17%). MRL values were exceeded most often in peppers (19%), followed by melons (6.1%), cauliflower (3%) and wheat grains (0.5%) (E.C, 2001).

A report from the UK government sponsored monitoring program indicated that, in the food samples analyzed: pesticide residues were detected in 34.8% of the samples with 33.1% being below the MRL whilst 1.7% was above the MRL (PRC 2006).

2.10 GENERAL CHARACTERISTICS OF LETTUCE PLANT

Lettuce (*Lactuca sativa*) is a [temperate annual](#) or [biennial plant](#) of the daisy family [Asteraceae](#). It is most often grown as a [leaf vegetable](#). It is eaten either raw, notably in [salads](#), [sandwiches](#), [hamburgers](#) and many other dishes, or cooked, as in [Chinese cuisine](#) in which the stem becomes just as important as the leaf. The lettuce plant has a short stem initially, but when it gradually blooms, the stem and branches lengthen and produce many flower heads that look like those of [dandelions](#), but smaller. This is referred to as [bolting](#). When grown to eat, lettuce is harvested before it bolts. Lettuce is used as a food plant by the [larvae](#) of some [Lepidoptera](#) (Hamilton, 2005). Appendix 6 – plate I shows the lettuce plant grown on the farm.

2.10.1 NUTRITIONAL AND MEDICINAL VALUES OF LETTUCE

As in most vegetables, the greener the leaves, the greater the nutrient content and a good source of chlorophyll. Lettuce has very low calorie content and is composed primarily of

water, about 90-95%. It also contains fiber, minerals (potassium, calcium, phosphorous, iron and magnesium); anti-oxidants such as beta-carotene and vitamins A, C, E and K, folic acid, as well as many vitamins of the B complex. The milky latex, found mainly in the stems and leaf nodes of lettuce, contain a mixture of active principles which have been used for centuries for its medicinal virtues. It has extraordinary sedative and painkilling properties similar to those of opium. Although the milky latex is mostly found in the wild varieties of lettuce, commercial lettuce does contain it as well (Grigson, 1978; Hamilton, 2005).

The healthy properties of lettuce are immense due to its many nutrients and anti-oxidants found in the leaves. According to Mandora (2010), these are:

- Anti-anemic: Lettuce contains a relatively high amount of chlorophyll and iron, which are essential for the synthesis of hemoglobin in blood red cells.
- Anti-oxidant: Lettuce is rich in anti-oxidants, especially beta-carotene, vitamin C and vitamin E. These substances help cleanse the body from toxins, prevent the damage caused by free-radicals, prevent premature aging and lower the risk of chronic diseases and cancer.
- Bone health: Lettuce has a protective effect on bone health, due to its high content in vitamin K which is required for the synthesis of osteocalcin, a bone protein which helps strengthen bone tissue. This bone protective action is particularly effective in preventing osteoporosis-related fractures in post-menopausal women and older adults. Lettuce is a good source of calcium and phosphorous, as well, which play a key role in bone structure and health.
- Constipation: Lettuce has high fiber content and its juice can help stimulate the function and motility of the intestinal tract, relieve constipation and cleanse the colon.

- Cough: Lettuce juice contains anti-cough agents, which can help relieve irritable cough, as well as the symptoms of asthma and bronchitis.
- Hydration: Lettuce juice is an excellent source of hydration at the cellular level. They are refreshing, thirst-quenching and help rehydrates our cells and releasing toxins and harmful fats from our congested cells.
- Purifying: The purifying effect of lettuce is the result of the combined actions of anti-oxidants (which help neutralize harmful substances accumulate in the body), potassium (which promotes diuresis, thereby helping eliminate toxins from the body) and fiber (which helps cleanse the colon).
- Sedation: Lettuce latex is been used by physicians as a substitute for opium, due to its natural sedative and painkilling properties. And its medicinal use is now limited to treat sleep disorders, nervous excitability, anxiety and restlessness, especially in children and elderly persons.

The amount of nutrients present in 100 g of lettuce are as follows: Carbohydrates - 2.2 g, Dietary Fiber - 1.1 g, Fats - 0.2 g, Protein - 1.4 g, Water - 96 g, Vitamin A - 166 µg, Folate (Vitamin B9) - 73 µg, Vitamin C - 4 mg, Vitamin K - 24 µg, Iron - 1.2 mg.

2.10.2 PESTS AND DISEASES OF LETTUCE

There are various pests and diseases that affect the growth and development of lettuce plant. These pests and diseases attack lettuce at various stages in the growth of the plant. The notable pests of lettuce are Aphids, Corn Earworm (*Heliothis sp.*) and Loopers (*Trichoplusa ni*). Diseases such as *Sclerotinia species*, *Bremia lactucae*, *Erysiphe cichoracearum*, *Marssoninia panattoniana*, *Rhizoctonia solani* affect the growth of lettuce plant (Lettuce Pest

Guide, <http://www.slhfarm.com/lettucepest.html>). Other problems of the lettuce plant that affect its growth are Tip burn and Rib blight (McDougall, 2006).

2.10.2.1 Aphids

Aphids are small, soft-bodied insects that grow up to 1 to 4 mm long. They are sap suckers and form colonies on the new shoots of a wide range of crops. Species range from yellow to green to black. Colonies include mostly wingless and some winged individuals (DAFWA, 2007). They are found on new stems and the underside of the leaf where they suck fluids from the plant leaving a honey dew substance behind. Leaves turn pale yellow. These tiny insects cause wilting on the plant. They damage lettuce crop by vectoring diseases and also cause physical damage from too much feeding. A curled, distorted leaf, stunted plants indicates the infestation of aphids. There are various types of aphids that colonize lettuce. The main aphids found on lettuce are: Green Peach Aphid (*Myzus persicae*), Lettuce Aphid (*Nasonovia ribisnigri*), and Lettuce Root Aphid (*Pemphigus bursarius*) (Lettuce Pest Guide, <http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.2 Corn Earworm (*Heliothis* sp.)

This worm causes boring into the head of lettuce which makes it a much more serious pest of lettuce. Once inside the head it is very difficult to control. The dusty brown adult moth lays its eggs singly on the leaves which develop a red to brown ring in 24 hours. The eggs darken before hatching. Corn earworm eggs are more round and ridged than the flattened loopers eggs. Corn earworms have three inner legs and do not loop when they move. The worms usually develop distinct stripes as they mature and vary in color from green to black. Corn earworms appear more hairy than loopers. They feed mostly on the center of the plant, damaging new inner leaves. As the plant matures the corn earworms bore into the head

making the head unmarketable (Lettuce Pest Guide,<http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.3 Loopers (*Trichoplusa ni*)

Loopers are greenish worms lacking the inner legs between the front legs and back prolegs requiring them to loop or arch their middles to move. The brown dusty adult moths lay domed-shaped eggs singly on mostly the underside of leaves. The first instar feeds mostly on the underside of the leaves and just eats the surface. Later instars may move into the plant, eat holes in the leaves and leave greenish brown feces. This worm causes significant damage annually to lettuce in most growing areas. They cause problems in damaging leaves and causing food quality problems (Lettuce Pest Guide,<http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.4 Lettuce Drop (*Sclerotinia* sp.)

Lettuce drop is caused by two fungal pathogens, *Sclerotinia minor* and *Sclerotinia sclerotiorum*. Both species form survival structures known as sclerotia which are dark masses of fungi hyphae (the non-reproducing filamentous portion of fungi). Sclerotia can survive in soil for multiple seasons. When cool, moist soil conditions prevail, *Sclerotinia* is able to infect the stems and lower leaves that are in contact with the soil. Initial symptoms are a brown soft decay of the infected tissue. This infection can migrate toward and eventually kill the plant crown. Once the plant crown is infected, the plant will wilt and collapse. Plant collapse commonly occurs close to harvest when plants are at or near maturity. Under the dying plant, a cottony white mycelium with hard small black sclerotia that look like mouse turds is observed. Lettuce tissues that have wilted due to *Sclerotinia* infection will often have

solid brown to black sclerotia present on the surface. These can be distributed into the soil profile and persist to infect future lettuce crops. Sclerotia of *S. sclerotiorum* average ¼ inch in diameter while those of *S. minor* are generally 0.1 inch or smaller. (Lettuce Pest Guide, <http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.5 Downy Mildew (*Bremia lactucae*)

This is a disease that affects the growth of lettuce. The disease attacks the leaves causing a light green to chlorotic areas with white spores in these areas on the underside of the leaves. The spores are produced on branched stalks that look like trees. The lesions are angular and tend to be confined by the veins. As the disease progresses the lesions enlarge and can turn necrotic in which case oospores may be formed. All stages of the plant are susceptible. Mildew seldom kills the plant except if infection occurs heavy on the cotyledons or early leaves. Early infections can result in systemic infection in which case the vascular tissue turns brown to dark brown (Lettuce Pest Guide, <http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.6 Powdery Mildew (*Erysiphe cichoracearum*)

The disease can reduce quality, but it is not commonly economically important. Powdery mildew produces whitish sporulation like downy mildew, but it can occur on the upper as well as the lower side of leaves and powdery mildew spores are produced on chains on a single stalk. Sometimes powdery mildew spores produce small black fruiting bodies. Powdery mildew spreads in a circular pattern spreading a dusty growth over the whole leaf (Lettuce Pest Guide, <http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.7 Antracnose (*Marssoninia panattoniana*)

The disease appears as holes on the surface of the leaves and is therefore also called shothole. This disease starts as small, yellow, water-soaked spots on the lower leaves that darken, enlarge slightly and then dry up. After a while the centers fall out leaving small holes in the leaves. Affected leaves wilt and die. Cool temperatures and free moisture are required for disease spread (Lettuce Pest Guide, <http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.2.8 Bottom Rot (*Rhizoctonia solani*)

Bottom rot can cause serious losses in lettuce. Lesions occur on the lower leaves in contact with the soil, stem and lower midribs. The lesions appear as small sunken rust to brown colored spots. If conditions are right these spots can enlarge rapidly to rot leaf midribs and leaf blades. Amber coloured droplets sometimes ooze from infected midribs. The disease then can spread up the head. The entire head can rot and die from this disease, although its usually a quality problem (Lettuce Pest Guide, <http://www.slhfarm.com/lettucepest.html>) (8/9/2010).

2.10.3 OTHER PROBLEMS OF THE LETTUCE PLANT

2.10.3.1 Tip Burn

Tip burn can cause serious quality problems in marketing lettuce and are difficult to control since the cause is not well understood. Symptoms are not apparent on the outside of the head. Symptoms become apparent when the head is broken and also on the inner leaves. Vein discoloration and brown to black spots near the leaf margins indicates the symptoms of tip burn. Tip burn is thought to be a result of calcium deficiency in the plant. Applying additional calcium does not always solve this problem and it can occur on calcium rich soils. Difficulty in calcium uptake by plants may be the problem. Foliar calcium applications may help. Warm

temperatures, rapid growth, late fertilization are some of the possible causes of tip burn (McDougall, 2006).

2.10.3.2 Rib Blight

This is also called brown rib and is characterized by brown to black discoloration of the lower midrib and vascular branches off the midrib. Rib Blight tends to occur when periods of cool weather are followed by hot weather. Some varieties of lettuce are more susceptible than others (McDougall, 2006).

2.11 PRE-HOUSEHOLD PROCESSING AND TREATMENTS OF LETTUCE

Household or commercial food processing is the preparation of food using various mechanical processes, such as removing damaged or soiled items or parts of crops, washing, peeling, trimming or hulling. This often leads to significant reduction in the amount of pesticide residues in the remaining edible portions (Petersen *et al.*, 1996). The extent to which pesticide residues are removed by household or commercial processing depends on a variety of factors, such as the chemical properties of the pesticide, the nature of the food commodity, the processing step and the length of time the compound has been in contact with the food (Holland *et al.*, 1994). Household processing and treatments procedures used to reduce pesticide residue on lettuce are washing with tap water, salt water washing and detergent washing.

2.11.1 WASHING UNDER RUNNING TAP WATER

Household washing procedures are normally carried out with running or standing water at moderate temperatures. Detergents, chlorine or ozone can be added to the wash water to improve the effectiveness of the washing procedure (Ong *et al.*, 1996). If necessary, several washing steps can be conducted consequently.

The effects of washing depend on the physiochemical properties of the pesticides, such as water solubility, hydrolytic rate constant, volatility and octanol-water partition coefficient (Pow), in conjunction with the actual physical location of the residues. Washing processes lead to reduction of hydrophilic residues which are located on the surface of the crops. In addition, the temperature of the washing water and the type of washing has an influence on the residue level. As pointed out by Holland *et al.*, 1994, hot washing and the addition of detergents are more effective than cold water washing. Washing coupled with gentle rubbing by hand under tap water for 1 min dislodges pesticide residues significantly. Systemic and lipophilic pesticide residues are not removed significantly by washing.

Ramesh and Balasubramanian (1999) performed a study with fruits and vegetables collected from Chennai local markets. The samples were fortified with known concentrations of various pesticides followed by decontamination study with different household preparations like washing and cooking. This resulted in 65-95% decontamination of pesticide residues of the raw market samples analyzed. Low levels of pesticide residues were detected in 97(40%) of 243 samples analyzed after following normal household washing, peeling and cooking procedures. The number of samples containing detectable residues dropped to 47(19%) after

household preparation. These results indicate that residue levels in most commodities are substantially reduced after household preparation (Schattenberg *et al.*, 1996).

2.11.2 WASHING WITH SODIUM CHLORIDE

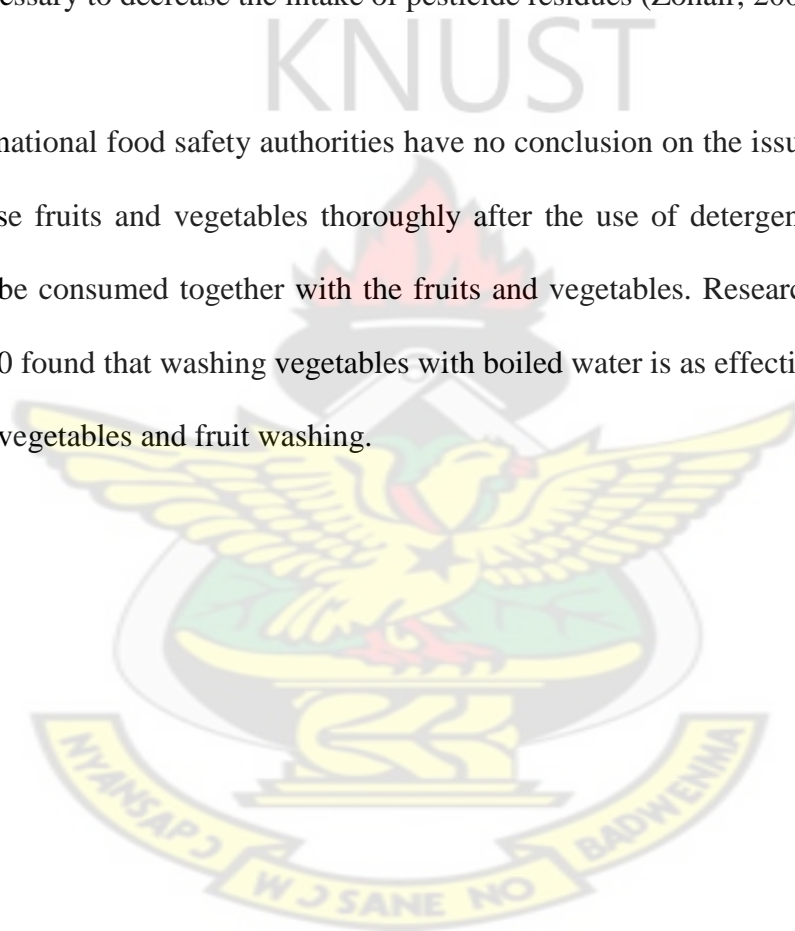
Sodium chloride solution is largely used to decontaminate the pesticide residues from different fruits and vegetables. There are several studies to prove the efficacy of salt water washing to dislodge the pesticides from crops. The chopped fruits and vegetables are put in a beaker containing 5% sodium chloride solution, after some minutes the plant samples are gently rubbed by hand in salt solution and the water is decanted. Salt solution is a better sanitizer at an appropriate concentration of 35 ppm and 2 min contact time compared with potable water. Efficacy improves with increasing temperature and increasing concentration however, higher concentration has a deteriorating effect on the appearance of some crops such as lettuce.

Kumari, 2008 reported that dipping of green chillies in 2% salt solution for 10 minutes followed by water wash proved to be effective. This resulted in the removal of 32.56 and 84.21% residues of triazophos at 0 and 5 days respectively after spray of triazophos. In that same study, the acephate residues were removed to an extent of 78.95% at zero day. Following same technique, Kumari, 2008 further observed 90.56 and 66.93% reduction correspondingly on 0 and 5 days after spraying of cypermethrin in chillies. In addition to sodium chloride (NaCl) solution, hydrochloric acid (HCl), acetic acid, sodium hydroxide (NaOH) solution and potassium permanganate (KMnO₄) have the ability to cause a reduction in the residues on vegetables.

2.11.3 WASHING WITH DETERGENT

Agricultural pesticides do not come off with water alone. Adding washing-up liquid (detergent) to water and generously swishing the fruit or vegetables around for a couple of minutes can often lift off much of the pesticide residue. Detergent helps to remove certain amount of pesticide residues on the surface of fruits and vegetables. Washing with detergent solutions is necessary to decrease the intake of pesticide residues (Zohair, 2001).

However, international food safety authorities have no conclusion on the issue. Nevertheless, you should rinse fruits and vegetables thoroughly after the use of detergent, otherwise the detergent may be consumed together with the fruits and vegetables. Research conducted by Krol *et al.*, 2000 found that washing vegetables with boiled water is as effective as using soap specialized for vegetables and fruit washing.



KNUST

CHAPTER THREE

MATERIALS AND METHODS

3.1 SCOPE OF STUDY

This research was organized in two phases. Phase I dealt with the administration of questionnaires to farmers to examine pesticide use pattern in the Kumasi Metropolis. The knowledge, attitudes and the practices of farmers were sought for in the first phase of this work. Vegetable farmers from two selected farm sites, Gyinyase and Quarters all in Kumasi were involved in this study.

Phase II dealt with analysis of chlorpyrifos residues on lettuce. It evaluates the impact of various household preparations procedures (washing with tap water, salt water washing and detergent washing) on chlorpyrifos residues on lettuce. It also assesses the dissipation rate of chlorpyrifos at the selected farm site. The Quarters farm site was used for this phase of the research.

3.2 STUDY AREA

This study was conducted on urban vegetable farm site in the Kumasi Metropolis, the capital of the Ashanti Region. Kumasi is the second largest city in Ghana. It has a population of about 1.2 million people and an annual growth rate of 5.9% (Ghana Statistical Service, 2002). It is located between latitude 6° 42 N and longitude 1° 35 W and lies approximately 260 m above sea level.

The city covers an area of 22,300 hectares. It has two main seasons; the major rainfall season occurring between March and September, peaking in June and August and the dry season occurring between November and March making rainfall bimodal within the city with mean annual rainfall of 1300 mm and mean temperatures of 28 °C. The relative humidity ranges between 1270 to 1410 mm with average daily sunshine durations ranging between 2 to 7 hours and daily maximum and minimum temperatures of 35.50 °C and 21.20 °C respectively (Meteorological Services Department, 2002).

Agriculture remains an important livelihood component for the urban residents and becoming more intensive as the urban area and populace grows. Production emphasis is shifting towards high value, perishable products which come with a ready urban market (Danso *et al.*, 2003).

3.3 SITE DESCRIPTION

Vegetable production farm site at Quarters was selected. This is among one of the largest urban vegetable farming site in Kumasi. It is located on the right side of the main stretch of road from Tech Junction to Atonsu. The site was selected based on higher farmer population

densities (assumed to have higher pesticide usage) and the time engaged in vegetable production. It is about 9 km from the centre of Kumasi. The farm covers a total area of 12 ha. The number of vegetable beds owned by each farmer ranged from 10 to 60 with an average bed size of 12 sq metre. All the farmers cultivate mainly lettuce, cabbage, spring onions, green pepper and carrots all year round. The field trial was conducted in the dry season from October – December, 2010 through a farmer who agreed to donate his plot for the participatory experimentation. An overview of the Quarters farm site is presented in appendix F, plate II.

3.4 SAMPLING

Fresh samples of lettuce were collected at different time intervals after spraying and at different growth stages from the field to assess the residue levels of chlorpyrifos on the crop. Samples were collected at various growth stages or periods of the lettuce. Growth stages depended on the weeks taken for the folding of lettuce leaves. At each of the growth stages (first, second and third growth stages), samples of lettuce were randomly collected from the farm at different time intervals after the application of insecticides. The samples were taken at 0 day (1 hour after application), 1 and 7 days after insecticide application. Two (2) samples of lettuce were collected at each sampling period. A total of 72 samples of lettuce were used for this work (thus, 3 growth stages x 3 different time intervals (days) after the application of insecticides x 8 samples per each day). Samples were wrapped in aluminum foil and transported to the laboratory immediately after collection and stored at – 4 °C until analysis. Samples were then cut into small pieces, mixed thoroughly and sub-samples were weighed for each pre-household treatment procedure. Samples were subjected to three (3) pre-household treatment procedures and one (1) unprocessed or control treatment. Thus each

sample collected at each sampling day was divided into four, for each of the treatments and labeled as T_0 , T_1 , T_2 and T_3 .

3.5 PRE-HOUSEHOLD TREATMENT OF SAMPLES

The samples collected from the farm were subjected to various pre-household treatment procedures. Three (3) pre-household treatment procedures and one (1) unprocessed or control sample were conducted. In treatment one, each sub-sample (100 g) was washed under running tap water for 2 minutes (T_1). In the second treatment, the fresh samples were dipped in 500 mL 2% salt solution at room temperature, (T_2) condition for 2 minutes and washed under tap water for 2 minutes. In the third treatments, this procedure was applied using 1% detergent solution at room temperature (T_3) for 2 minutes followed by 2 minutes wash under running tap water. The field samples that were analyzed without any household treatment procedure were designated as unprocessed control (T_0).

3.6 EXPERIMENTAL PROCEDURE

Extraction of pesticide residues from lettuce, their detection and quantification by analytical techniques are the major steps involved in pesticide residue analysis.

3.6.1 EXTRACTION OF PESTICIDE RESIDUE FROM LETTUCE

A critical review of literature showed that different solvents such as n-hexane, petroleum ether, methylene chloride and acetone or ethyl acetate have been used for the extraction of pesticide residue from vegetables (Pihlström *et al.*, 2007). Startin *et al.*, 2000 found that ethyl acetate has proved to be a good solvent compared with other solvents for the extraction of

several pesticides from vegetables because its polarity is high and it is a less volatile and thermally labile compound.

In the present study, FAO/ WHO 1996 procedure for extraction of pesticide residues was followed with little modification. About 10g of the lettuce was homogenized in a mortar and transferred to a pre-cleaned extraction thimble. This was extracted with 100 ml ethyl acetate for 8 h in a soxhlet apparatus cycling 4-5 times per hour (appendix F- plate IV shows soxhlet apparatus in use). The extract was then concentrated using a rotary evaporator prior to analysis (appendix F – plate V shows rotary evaporator in use).

3.6.2 ANALYTICAL TECHNIQUE USED FOR RESIDUE ANALYSIS

An aliquot (1 μL) of the extract was injected into gas chromatograph with 10 μL Hamilton Syringe. The residues of chlorpyrifos were analyzed on GC (Agilent Technologies 6890N Network GC system) with electron capture detector (ECD-Source Ni^{63}) coupled with Chemito 5000 data processor. The HP-5 capillary column (30 m x 0.32 mm i.d.) of 0.25 μm film thickness was used. The temperatures were: Oven 210 $^{\circ}\text{C}$, Injector 230 $^{\circ}\text{C}$, Detector 300 $^{\circ}\text{C}$. Flow rate of carrier gas (Nitrogen, purity 99.97%) is 2 mL min^{-1} and make up gas (Nitrogen, purity 99.97%) is 60 mL min^{-1} . The residues of chlorpyrifos in samples were identified by comparing the retention time of the sample peaks with the standard (99.5% purity) solution containing 1 ppm of chlorpyrifos. No independent method of confirmation was applied. Peak heights were used for the quantitative determination of residues.

3.6.3 QUALITY CONTROL MEASURES

Recovery analyses were carried out on samples fortified at 0.001 mg kg^{-1} by adding standard pesticide solution. The samples were allowed to equilibrate for 30 minutes prior to extraction. After extraction and solvent evaporation, the samples were analyzed according to the proposed method. The recovery values were calculated from calibration curves constructed from the concentration and peak area of the chromatograms obtained with standards of chlorpyrifos. The recovery of the pesticide was in the range between 80 and 110%. Detection limits of the method were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC. Blank analyses were also performed in order to check interference from the sample. Concentrations of the residue in each sample were recorded in mg kg^{-1} and the average concentration of each pesticide was compared to the FAO/WHO (2004), Maximum Residue Level (MRL) value of 0.05 mg kg^{-1} . For quality control of the gas chromatographic conditions, a checkout procedure was performed before sample analysis.

3.7 ADMINISTRATION OF QUESTIONNAIRE

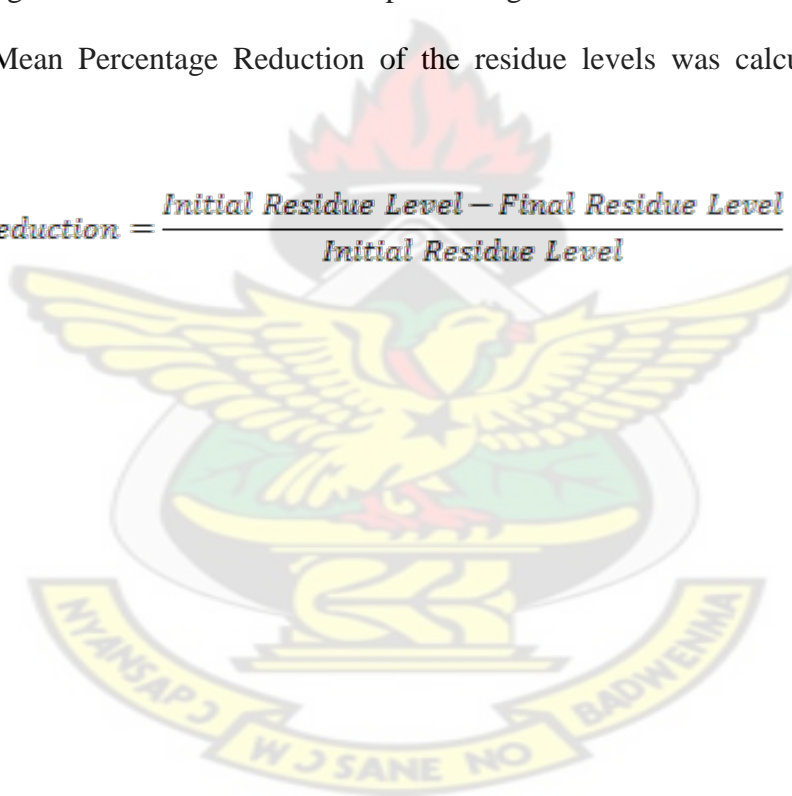
Formal interviews and field observations were used to gather information on the type, scope and extent of use of pesticide, farmers' knowledge of pesticides and their perceptions about the chemicals potential to harm. In the formal interviews, structured questionnaires were used to gather information from farmers. The questionnaires were administered to forty-eight (48) farmers. All questions were closed questions in a multiple-choice format, so that respondents only tick the appropriate answer. Some questions also demanded multiple answers. The questionnaires were administered on the farms and in most cases; the investigator translated the questionnaires into easily understandable language to get the original meaning. In addition to the interviews, field observation survey was discreetly conducted. The farmers

were not informed before hand in order to avoid modifications in pesticide use and management practices and to reduce investigator/respondent bias.

3.8 STATISTICAL ANALYSIS

The results from the Phase I of the research were analyzed using Microsoft Excel and that for Phase II were analyzed using Sigma Plot One-Way Analysis of Variance. The Student-Newman-Keuls (SNK) Method was used as a multiple comparison procedure to determine the statistical significance of various home processing treatments in reducing chlorpyrifos residues. The Mean Percentage Reduction of the residue levels was calculated using the relation:

$$\text{Percentage Reduction} = \frac{\text{Initial Residue Level} - \text{Final Residue Level}}{\text{Initial Residue Level}} \times 100\%$$



CHAPTER FOUR

RESULTS

4.1 PERCEPTION, ATTITUDES AND KNOWLEDGE OF FARMERS IN THE STUDY AREA

4.1.1 GENERAL INFORMATION AND EDUCATIONAL BACKGROUND

Lettuce, cabbage, spring onion, and green pepper are cultivated simultaneously in the area. Some of the respondents had been cultivating vegetables for over 12 years and this has been their main source of livelihood. The spouses of most of the male farmers also sell the vegetables after harvesting. The farm size of each of the vegetable farmers was found to be dependent on the relative time (years) they have been engaged in vegetable cultivation. The financial position of the farmer also determined the size of the farm. Some farmers use hired labour on their farms. From the results presented in Table 4.1, it was realized that 25% of the respondents have had no formal education. Moreover, 75% of the farmers have had some level of education. Out of these about 45.8% of the farmers have had education up to the basic level whiles 29.2% have been educated up to the secondary school level.

Table 4.1: Educational level of farmers in the study area

Variables	Quarters [%]		Gyinyase [%]		Total	[%]
No official education	8	[33.3]	4	[16.7]	12	[25.0]
Primary	11	[45.8]	11	[45.8]	22	[45.8]
Secondary (Agric School)	5	[20.8]	9	[37.5]	14	[29.2]

Tertiary	0	[0.0]	0	[0.0]	0	[0.0]
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4.1.2 MODE OF SELECTION OF PESTICIDE BY FARMERS

All respondents use pesticide to control pest on their vegetables. The efficacy, level of safety and the availability of the insecticide were found to be the main factors in the selection of the insecticide for use. The main factor informing the selection of insecticide product to control pest was its efficacy. The second important factor informing farmers on the pesticide choice was the level of safety of the pesticide. The availability of the pesticide on the market was the third important factor in selecting a pesticide for use.

From the results presented in Table 4.2, it was realized that 66.7% and 45.8% of farmers at Quarters and Gyinyase farms respectively said they considered the efficacy of the insecticide before selecting it for use. 20.8% and 37.5% of Quarters and Gyinyase respondents respectively also found the level of safety of the insecticide an important factor in the selection of the insecticide for use. The other important factor was found to be the availability of the insecticide on the market with 12.5% and 8.3% of Quarters and Gyinyase farmers respectively attesting to this fact. Cost of the insecticide was not an issue bothering the farmers. This is because, farmers expect good yields on their farms and therefore purchase potent pesticides without taking into consideration the cost.

Table 4.2: Factors for selection of pesticides by farmers in the study area

Variables	Quarters [%]		Gyinyase [%]		Total [%]	
Efficacy	16	[66.7]	11	[45.8]	27	[56.2]
Level of Safety	5	[20.8]	9	[37.5]	14	[29.2]
Availability	3	[12.5]	2	[8.3]	5	[10.4]
Cost	0	[0.0]	2	[8.3]	2	[4.2]

4.1.3 SOURCE OF INFORMATION ON PESTICIDE SELECTION

Most pesticides were obtained from pesticide retailers, who supplied respondents with pesticides. Information on pesticides and their use was obtained from extension officers, fellow farmers, media (radio announcements) and in some cases through personal experiments that are conducted by the farmers on their own. Pesticide labels played no significant role in serving as a source of information to respondents.

From the results presented in Table 4.3, it was realized that extension officers provided information to 54.2% and 58.3% of Quarters and Gyinyase farmers respectively. Fellow farmers provided information to 25% and 20.8% of Quarters and Gyinyase farmers respectively. Whilst 16.7% of the respondents from the Quarters farms relied on personal experiments, 12.5% of Gyinyase respondents relied on personal experiments which are carried out on their own to determine the efficacy or otherwise of the pesticide.

Table 4.3: Source of information on pesticide selection

Variables	Quarters [%]	Gyinyase [%]	Total [%]
Extension Officers	13 [54.2]	14 [58.3]	27 [56.3]
Fellow farmers	6 [25.0]	5 [20.8]	11 [22.9]
Media (Radio announcement)	1 [4.2]	2 [8.3]	3 [6.3]
Pesticide label	0 [0.0]	0 [0.0]	0 [0.0]
Personal experiments	4 [16.7]	3 [12.5]	7 [14.6]

4.1.4 APPLICATION PRACTICES OF FARMERS IN THE STUDY AREA

Farmers from the Gyinyase and Quarters areas used more than one pesticide product per crop cycle (approximately two months). Of these farmers, over 72% stated that they applied these pesticide products in mixtures of two or more. It was also realized that application of the pesticide product in single or in combination depends on the vegetable under cultivation. According to the survey, in cultivation of lettuce, only one pesticide active ingredient is used whilst in the cultivation of cabbage, it involves the use of two pesticide products.

4.1.5 ESTIMATION DOSE OF PESTICIDES DURING SPRAYING

Two different measuring cups (lid of pesticide container and 15ml measuring cup) were used to estimate the amount of pesticide to use. From the results presented in Table 4.4, it was realized that 50% and 45.2% of respondents in the Quarters and Gyinyase farms areas respectively relied

on the lid of the pesticide container as a means of measuring the pesticides to be used during spraying. 37.5% and 33.3% of respondents in the Quarters and Gyinyase farms respectively said they relied on experience to measure the amount of pesticide to be applied. 12.5% of the respondents in the Gyinyase and Quarters farms all used the 15ml measuring cup to estimate the pesticide dose to be applied on the vegetable.

Most farmers (52.1%) use the lid of the pesticide container to measure the volume of pesticide to be used while 35.4% of the farmers also relied on personal experience to estimate the doses of the pesticide to use. They could not state precisely the quantity of water added and the land area over which the product is applied. 12.5% of the farmers also used the 15 ml measuring cup to estimate the dose of pesticide to be used. Farmers could not state precise application doses to use for specific pests and crop stages. According to farmers, higher tank doses were used during the first pesticide treatment of the season (mostly during the seedling stage) and during heavy pest infestation.

Table 4.4: Methods for estimating the volume of pesticide

Variables	Quarters [%]		Gyinyase [%]		Total [%]	
Lid of pesticide container	12	[50.0]	13	[45.2]	25	[52.1]
Experience	9	[37.5]	8	[33.3]	17	[35.4]
15 ml measuring cup	3	[12.5]	3	[12.5]	6	[12.5]
Others	0	[0.0]	0	[0.0]	0	[0.0]

4.1.6 PROTECTION OF FARMERS DURING AND AFTER PESTICIDE

APPLICATION

Farmers applied pesticides (insecticides) themselves using hand operated CP-15 Knapsack sprayer. Knapsack sprayer was not own individually but it belongs to a group of farmers at each zone which is used at anytime a farmer needs it.

Farmers did not protect themselves from contamination during the application of pesticides. Standard personal protective clothing such as eye goggles, nose mask, wellington boots and overall coats were never worn by farmers during pesticide application. Instead normal farm cloths were worn by applicators during pesticide application (appendix F – plate II shows a picture of a farmer applying pesticide). A dangerously inadequate substitute for nose mask like handkerchiefs which was wrapped over the mouth and nostrils was found to be used by farmers during pesticide application. From the results presented in Table 4.5, it was realized that none of the farmers were fully protected during pesticide application. However, 27.1% of the farmers partially protected themselves during pesticide application while 72.9% had no protective cover during pesticide application.

Table 4.5: Extent of protective cover used by farmers

Variables	Quarters [%]		Gyinyase [%]		Total [%]	
No protective cover	19	[79.2]	16	[66.7]	35	[72.9]
Partial protective cover	5	[20.8]	8	[33.3]	13	[27.1]

Full protective cover	0	[0.0]	0	[0.0]	0	[0.0]
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4.1.7 RE-ENTRY PERIOD OF FARMERS AFTER PESTICIDE APPLICATION

The re-entry period for farmers onto their farms (beds) after they have applied pesticides was less than 24 hours. From the results presented in Table 4.6, it was realised that, 29.2% and 37.5% of Quarters and Gyinyase farmers respectively worked on their beds less than 24 hours after pesticide application. Again, 70.8% and 62.5% of Quarters and Gyinyase farmers respectively worked on their beds less than 48 hours after pesticide application. Within the second day (48 hours) after pesticide application, all the farmers do work on their farms. None of the farmers left their beds for more than 48 hours without working on them.

Table 4.6: Farmers re-entry period after pesticide application

Variables	Quarters [%]		Gyinyase [%]		Total [%]	
Less than 24 h	7	[29.2]	9	[37.5]	16	[33.3]
Between 24 and 48 h	17	[70.8]	15	[62.5]	32	[66.7]
From 48 to 72 h	0	[0.0]	0	[0.0]	0	[0.0]
More than 72 h	0	[0.0]	0	[0.0]	0	[0.0]

4.1.8 DISPOSAL OF EMPTY PESTICIDE CONTAINERS AND USED WATER

Farmers dispose off empty pesticide containers by principally throwing them away on the farm site. From the results presented in Table 4.7, it was realised that, 83.3% of the Quarters and 70.8% of Gyinyase respondents disposed the empty pesticide containers by throwing them on the

farm whiles 16.7% and 29.2% of Quarters and Gyinyase respondents burned the empty pesticide containers at the farm site. None of the respondents rinsed and used the containers as food or water receptacles nor even piled the empty pesticide containers with the intention of selling them to buyers.

Table 4.7: Method of disposal of empty pesticide containers by farmers

Variables	Quarters [%]		Gyinyase [%]		Total [%]	
Pile and Sell	0	[0.0]	0	[0.0]	0	[0.0]
Throw away on farm	20	[83.3]	17	[70.8]	37	[77.1]
Bury in ground at farm	0	[0.0]	0	[0.0]	0	[0.0]
Burn on farm	4	[16.7]	7	[29.2]	11	[22.9]
Food and water receptacles	0	[0.0]	0	[0.0]	0	[0.0]

However, water use to wash the CP-15 Knapsack sprayers was disposed off on the farm. But, it was observed on the field that, some of the farmers disposed the wash water either on the field or in the nearby water bodies depending on where the washing is done.

4.2 RESULTS OF CHLORPYRIFOS RESIDUE ANALYSIS ON *LACTUCA SATIVA*

By applying the recommended gas chromatography conditions, the retention time of chlorpyrifos residue in lettuce extracts were compared with that of a standard solution. The retention time of chlorpyrifos was 10.296 min. A sample of GC chromatograph for quantitative determination of

chlorpyrifos in lettuce leaves analyzed following the recommended procedure is presented in the appendix G.

4.2.1 CHLORPYRIFOS RESIDUE LEVELS ON CONTROL SAMPLES (T_0)

In Table 4.8, the range, arithmetic mean and the standard deviation of the mean of chlorpyrifos residue levels detected in lettuce without any household preparation procedure (T_0) are presented. The detected residue levels observed were 0.059 ± 0.008 , 0.052 ± 0.0014 and $0.006 \pm 0.002 \text{ mg kg}^{-1}$ for 0 (1 hour), 1 and 7 days after pesticide application on the first stage during the growth of lettuce. In the second stage of growth of lettuce, the detected residue levels were 0.055 ± 0.0014 , 0.053 ± 0.012 and $0.007 \pm 0.0017 \text{ mg kg}^{-1}$ for 0 (1 hour), 1 and 7 days after pesticide application respectively. In the third stage, the detected residue levels were 0.055 ± 0.009 , 0.034 ± 0.003 and $0.002 \pm 0.00 \text{ mg kg}^{-1}$ for 0 (1 hour), 1 and 7 days after pesticide application respectively.

Table 4.8: Results of chlorpyrifos residue levels (mg kg^{-1}) of control samples (T_0) at different stages of growth, $n = 6$

Days	Stage 1	Stage 2	Stage 3
0	Range	0.053 – 0.065	0.054 – 0.056
	Mean \pm S.D	0.059 ± 0.008	0.055 ± 0.0014
1	Range	0.051 – 0.053	0.044 – 0.062
	Mean \pm S.D	0.052 ± 0.0014	0.053 ± 0.012

Range	0.0045 – 0.0075	0.0058 – 0.0082	0.002
Mean ± S.D	0.006 ± 0.002	0.007 ± 0.0017	0.002 ± 0.00

4.2.2 CHLORPYRIFOS RESIDUE LEVELS AFTER WASHING UNDER TAP WATER (T₁)

In Table 4.9, the range, arithmetic mean and the standard deviation of chlorpyrifos residue levels detected in lettuce after washing with tap water are presented. The detected residue levels of chlorpyrifos observed in the first stage during the growth of lettuce were 0.049 ± 0.009 , 0.048 ± 0.004 and 0.006 ± 0.0007 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively. During the second stage of growth of lettuce, the detected residue levels of chlorpyrifos observed were 0.052 ± 0.004 , 0.04 ± 0.007 and 0.005 ± 0.0028 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively. In the third stage, the detected residue levels of the pesticide that were observed on lettuce were 0.053 ± 0.0028 , 0.03 ± 0.0056 and 0.002 ± 0.0014 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively.

Table 4.9: Results of chlorpyrifos residue levels (mg kg⁻¹) in samples after treatment 1 (T₁) at different stages of growth, n = 6

Days	Stage 1	Stage 2	Stage 3
Range	0.042 – 0.056	0.0049 – 0.055	0.051 – 0.055
0			
Mean ± S.D	0.049 ± 0.009	0.052 ± 0.004	0.053 ± 0.0028
Range	0.045 – 0.051	0.035 – 0.045	0.026 – 0.034

Mean \pm S.D	0.048 ± 0.004	0.04 ± 0.007	0.03 ± 0.0056
Range	$0.0055 - 0.0065$	$0.003 - 0.007$	$0.001 - 0.003$

7

Mean \pm S.D	0.006 ± 0.0007	0.005 ± 0.0028	0.002 ± 0.0014
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4.2.3 CHLORPYRIFOS RESIDUE LEVELS AFTER SALT WATER TREATMENT

(T₂)

In Table 4.10, the range, arithmetic mean and the standard deviation of chlorpyrifos residue levels detected in lettuce after salt water washing are presented. The detected residue levels observed during the first stage of the growth of lettuce were 0.039 ± 0.0056 , 0.023 ± 0.004 and 0.004 ± 0.0004 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively. During the second stage of growth, the detected residue levels of chlorpyrifos on lettuce were observed to be 0.049 ± 0.008 , 0.03 ± 0.004 and 0.004 ± 0.0014 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively. The detected residue levels on lettuce that was observed during the third stage of the growth of lettuce were 0.051 ± 0.0014 , 0.025 ± 0.004 and 0.002 ± 0.0004 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively.

Table 4.10: Results of chlorpyrifos residue levels (mg kg⁻¹) in samples after treatment 2 (T₂) at different stages of growth, n = 6

Days	Stage 1	Stage 2	Stage 3
Range	$0.035 - 0.043$	$0.0043 - 0.055$	$0.050 - 0.052$
0			
Mean \pm S.D	0.039 ± 0.0056	0.049 ± 0.008	0.051 ± 0.0014
Range	$0.02 - 0.026$	$0.027 - 0.033$	$0.022 - 0.028$

1

Mean \pm S.D	0.023 ± 0.004	0.03 ± 0.004	0.025 ± 0.004
Range	$0.0037 - 0.0043$	$0.003 - 0.005$	$0.0017 - 0.0023$

7

Mean \pm S.D	0.004 ± 0.0004	0.004 ± 0.0014	0.002 ± 0.0004
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4.2.4 CHLORPYRIFOS RESIDUE LEVELS AFTER MILD DETERGENT

TREATMENT (T₃)

In Table 4.11, the range, arithmetic mean and the standard deviation of chlorpyrifos residue levels detected in lettuce after detergent washing are presented. The detected residue levels observed during the first stage of growth of lettuce was 0.015 ± 0.004 , 0.01 ± 0.004 and 0.003 ± 0.0008 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively. During the second stage of growth of lettuce, the detected residue levels observed were 0.048 ± 0.0098 , 0.027 ± 0.004 and 0.004 ± 0.0019 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively. The detected residue levels on lettuce that was observed during the third stage of the growth of lettuce were 0.048 ± 0.0056 , 0.019 ± 0.0098 and 0.001 ± 0.00028 mg kg⁻¹ for 0 (1 hour), 1 and 7 days after pesticide application respectively.

Table 4.11: Results of chlorpyrifos residue levels (mg kg⁻¹) in samples after treatment 3 (T₃) at different stages of growth, n = 6

Days	Stage 1	Stage 2	Stage 3
Range	$0.014 - 0.022$	$0.036 - 0.050$	$0.024 - 0.030$
Mean \pm S.D	0.018 ± 0.006	0.048 ± 0.0098	0.027 ± 0.003

0

	Range	0.007 – 0.013	0.024 – 0.03	0.012 – 0.026
1				
	Mean ± S.D	0.01 ± 0.004	0.027 ± 0.004	0.019 ± 0.0098
	Range	0.0024 – 0.0036	0.0026 – 0.0054	0.0008 – 0.0012
7				
	Mean ± S.D	0.003 ± 0.0008	0.004 ± 0.0019	0.001 ± 0.00028

4.2.5 MEAN PERCENTAGE REDUCTION OF CHLORPYRIFOS RESIDUES ON CONTROL SAMPLES

Chlorpyrifos residue levels on control samples were observed to reduce with time. During the first stage, the residue level observed after samples were collected 0 day (1 hour) after pesticide application was 0.059 mg kg⁻¹, which represents the initial residue level. The residue levels reduced to the final level of 0.052 mg kg⁻¹, 1 day after pesticide application and that caused a percentage reduction of 11.9% (Table 4.12) on chlorpyrifos residue levels on lettuce. The percentage reduction was calculated using the relation:

$$\text{Percentage Reduction} = \frac{\text{Initial Residue Level} - \text{Final Residue Level}}{\text{Initial Residue Level}} \times 100\%$$

The same calculations were done for the other values to obtain the percentage reduction values.

Table 4.12: Mean percentage reduction of chlorpyrifos residues after different sampling times (days) on control samples

Residues levels in mg kg ⁻¹ at different stage of growth				
Days	1	2	3	Mean % Reduction

0	0.059	0.055	0.055	-
1	0.052	0.053	0.034	17.9
	[11.9]	[3.8]	[38.1]	
7	0.006	0.007	0.002	91.1
	[89.8]	[87.2]	[96.3]	

[] - Percentage reduction

4.2.6 MEAN PERCENTAGE REDUCTION OF CHLORPYRIFOS RESIDUES AFTER VARIOUS PRE-HOUSEHOLD TREATMENT PROCEDURES

The mean of the detected residue levels in all the pre-household treatment procedures were calculated and are presented in Table 4.13. All the pre-household treatment procedures caused a reduction of chlorpyrifos residue levels on lettuce. The percentage reduction was calculated using the relation:

$$\text{Percentage Reduction} = \frac{\text{Initial Residue Level} - \text{Final Residue Level}}{\text{Initial Residue Level}} \times 100\%$$

For instance, in calculating the percentage reduction of chlorpyrifos residue levels in samples which were collected 0 day (1 hour) after pesticide application and treated with mild detergent (T₃), the initial residue level of 0.056 mg kg⁻¹ and the final residue level of 0.031 mg kg⁻¹ were used for the calculation to obtain the percentage reduction value.

Table 4.13: Mean percentage reduction of chlorpyrifos residue levels after various pre-household treatment procedures

Treatments	Residues in mg kg ⁻¹ at different day's intervals			Mean % reduction
	0	1	7	
T ₀	0.056	0.046	0.005	-
T ₁	0.051 [8.9]	0.039 [15.2]	0.004 [20]	14.7
T ₂	0.046 [17.9]	0.026 [43.5]	0.003 [40]	33.8
T ₃	0.031 [44.6]	0.019 [58.7]	0.002 [60]	54.4

[]-Percentage reduction

CHAPTER FIVE

DISCUSSIONS

5.1 IMPLICATIONS OF SURVEY RESULTS

The educational level of the farmers is low and they are not well informed on the health and environmental hazards of pesticides. The farmers therefore use pesticides at any stage of the crop without any awareness of the residues levels and their effects on human and environmental health. Farmers could not state precise application doses to be used for a specific pest and at what stage of growth of crop to be sprayed. They could not also state the number of beds (area) of application with specific quantity of pesticides. Due to this, there is the likelihood of over-dose application of pesticide on crop which could lead to accumulation of pesticide and increase the residue level of the pesticide on the cultivated crops. Under-dose application of pesticide could also lead to increased levels of the pests in the farm. From an open discussion it was observed that, most of the farmers did not follow GAP because of their ignorance or lack of such training. They did not follow the withholding periods, which is the minimum time you must wait between applying an agrochemical and final harvesting of the crop. This leads to increasing the residue levels of the pesticides on crops when they are consumed.

Pests and diseases is a serious problem that affect the yield of lettuce at the farm and all respondents in the present survey sprayed their crops with pesticides (insecticides) to control pests and diseases on lettuce. Pesticide application was done to improve the yield of the crops to enhance profitability as vegetable production served as the main source of income for most respondents at the area. Farmers in this study sprayed a wide range of pesticides on all their crops. Insecticides such as Attack (Emamectin benzoate), Pyrinex (Chlorpyrifos), Golan (Acetamiprid) were used to spray lettuce, cabbage and spring onions. All the insecticides found in this study are used for the purposes intended for and that there are no obvious indications of public health problems.

The application of mixtures of pesticide products is a common practice by both the Quarters and Gyinease farmers, which is in accordance with the work by Kotey *et al.*, 2008. This is fuelled by perception that pesticide mixtures are more powerful and effective than individual products which is not always the case (Jipanin *et al.*, 1997). The practice of using indiscriminate combinations of pesticides, particularly insecticides, contributes to the increase in incidences of insect pest infestation (Biney, 2001). Chemicals that act similarly (e.g. cholinesterase-inhibiting pesticides) can demonstrate additive toxicity even if, individually, they are below levels considered dangerous (EJF, 2002). The practice defies some of the basic principles of insecticide management. For instance, Metcalf (1980) in his recommendation of strategies for pesticide management, states that the use of mixtures of insecticides must be avoided, since mixtures of insecticides generally result in the simultaneous development of resistance.

Interactions with farmers showed that for cultivation of lettuce the insecticides Golan (Acetamiprid) and Attack (Emamectin benzoate) were being used in combination. Golan belongs to the Neonicotinoid group of pesticide and acts as an acetylcholine agonist while Attack belongs to the Avermectins and acts as a chloride channel activator. In this case, the modes of action of these two pesticide products are different and therefore enhance the activity of eliminating pests and diseases on lettuce.

The Knapsack sprayer was the only spraying equipment used by all the farmers and none of the farmers used motorized sprayers. The knapsack sprayer was not individually owned by the farmers. Lack of capital was the main reason why individual farmers did not own their own sprayers. The Knapsack sprayer belonged to a group of farmers who would use it at the time of spraying crops. The use of the knapsack sprayer in itself presents some dangers to the users, since it is prone to leakage, especially as the sprayer ages. Matthews *et al.*, 2003 identified causes of leakage from the knapsack and emphasized the need to provide better-quality equipment at an acceptable cost that will be more durable in a hot and humid tropical environment such as Africa. During spraying, farmers do not distinguish between target and non-target crops. The number of spray per crop season however varied widely among crops and the farmers interviewed in the survey. For instance, most lettuce farmers sprayed insecticides on the crop 3-4 times in 7 days intervals before the lettuce is harvested.

Information on the efficacy, level of safety, availability and cost of the pesticide as well as its choice for use by farmers comes mainly from extension officers and fellow farmers. Radio advertisements play no significant role in giving information on pesticides to the farmers.

Pesticide label played no role in informing farmers about the pesticide to choose to fight pests and diseases on their vegetables. This could be because most of the farmers are not able to read and understand the labels on the pesticide product.

Farmers failed to protect themselves from contamination during pesticide application. Standard protective clothing such as goggles, nose masks or overalls was never worn and this could impact negatively on the health of the farmers. Chlorpyrifos which was used in this study can be absorbed through all routes of exposure. Therefore if farmers failed to protect themselves during application of the insecticide, negative side effects associated with applying chlorpyrifos such as bloody or runny nose, coughing, chest discomfort, difficult or short breath, eye irritation would be experienced and that would impact negatively on the health of farmers. Clarke *et al.*, 1997 reported such cases of negative health impacts to farmers after being exposed to insecticides. Some farmers used handkerchiefs as substitutes for nose masks which is a dangerous act because the handkerchiefs are used for other purposes such as wiping the face and hands soon after pesticide application without washing.

The commonest way of disposing of sprayer wash water and empty pesticide container among the farmers interviewed was by throwing them on farm. The empty pesticide containers were commonly seen lying about when one walks on the field. Children could be at high risk when they are neglected and allowed to roam at the farm. None of the farmers piled and sold the empty pesticide nor used it as food or water receptacle.

5.2 CHLORPYRIFOS RESIDUE ANALYSIS

The detected residue levels of chlorpyrifos in some of the samples of lettuce were above the Maximum Residue Level (MRL) of 0.05mg kg^{-1} (FAO/WHO, 2004). Samples of lettuce which were analyzed without any pre-household treatment procedure/ control samples (T_0) had 55.6% of the residue levels above the MRL. This is an agreement with the observation made by Amoah *et al.*, 2006, where residues of chlorpyrifos in lettuce were above the MRL value. The detected residue levels were high in the control samples for 0 (1 hour) and 1 day after pesticide application in all the stages but the detected levels were relatively low after samples were harvested on the 7th day after pesticide application.

Shortly after applying chlorpyrifos on lettuce, the levels of total detected residue were markedly higher in the lettuce leaves due to the foliar application of the insecticide. The insecticide has direct contact with the leaves and remains on the leaves. This is evident from the results since the highest detected residue level of $0.059 \pm 0.008\text{ mg kg}^{-1}$ (Table 4.8) was observed 1 hour after application of the insecticide. After each application of the pesticide, leaves pesticide concentration was increased but decreased again in the course of the subsequent sampling time (days). The decrease in the residue concentration of chlorpyrifos may be due to one or more of factors such as volatilization or photodegradation. This is because, photodegradation may lead to products that also volatilize. Chlorpyrifos taken up by plants through leaf surfaces is usually lost through volatilization (Roberts and Hutson, 1999).

According to Smith (1968), chlorpyrifos when exposed to sunlight, undergoes hydrolysis in the presence of water to liberate 3, 5, 6-trichloro-2-pyridinol (TCP), which undergo further

decomposition to diols and triols and ultimately cleavage of the ring to fragmentary products. This shows that photodegradation is a factor to cause a decrease in the residue concentration of chlorpyrifos.

A gradual and continuous deterioration of the chlorpyrifos residues on the lettuce leaves were observed as a function of time (days) after pesticide application. There was a reduction in the detected residue level of chlorpyrifos with time. Dissipation of chlorpyrifos took place causing a reduction in the detected residue levels that was observed one (1) hour after pesticide application. After one (1) hour of application of chlorpyrifos, the mean detected residue levels were $0.059 \pm 0.008 \text{ mg kg}^{-1}$, $0.055 \pm 0.0014 \text{ mg kg}^{-1}$ and $0.055 \pm 0.009 \text{ mg kg}^{-1}$ for the first, second and third growth stages respectively in the control samples. After 1 day, the mean residue levels reduced to $0.052 \pm 0.0014 \text{ mg kg}^{-1}$, $0.053 \pm 0.012 \text{ mg kg}^{-1}$ and $0.034 \pm 0.003 \text{ mg kg}^{-1}$ for the first, second and third growth stages respectively. There was a mean percentage reduction of 17.9% of initial residue levels detected 1 hour after pesticide application (Table 4.12). After 7 days of pesticide application, the detected residue levels further reduced to $0.006 \pm 0.002 \text{ mg kg}^{-1}$, $0.007 \pm 0.0017 \text{ mg kg}^{-1}$ and $0.002 \pm 0.00 \text{ mg kg}^{-1}$ for the first, second and third growth stages respectively. There was a mean percentage reduction of 91.1% of initial residue levels detected 1 hour after pesticide application (Table 4.12).

Stage of growth at which treatment is applied is an important factor that affects insecticide deposition and residue dissipation of insecticide on a crop. The differences in detected residue levels among lettuce across the various stages of growth were due to different plant sizes. Bigger lettuce plants with larger leave surface area traps large amount of the pesticides and more

sunlight compared to smaller lettuce plants. There is no data on the amount of pesticide trapped or the amount of sunlight trapped by the leaves during each stage of growth of lettuce. However, the detected residue levels on the first stage of growth in this study were relatively higher in most cases than in the second and the third growth stages. Therefore, the larger surface area of the leaves during the third growth stage might have contributed to the higher dissipation rate of chlorpyrifos by absorbing more sunlight from the environment which speeds up the rate of photodegradation. The larger surface area of the leaves also enabled higher volatilization of the insecticide at that stage of the growth of lettuce. Also, the highest mean percentage reduction of 38.1% and 96.3% on the 1st and 7th day respectively on the control samples were observed on the third growth stage.

Environmental conditions such as temperature influenced pesticide dissipation on plant through volatilization and photodegradation. Obviously, a higher temperature tends to favour volatilization and photodegradation of pesticides from plants, because the vapour pressure of the pesticide compound is temperature-dependent and additionally the adsorption to the leaf surface decreases with increasing temperature.

From the results of ANOVA presented in appendix B, the values obtained for control samples (T_0) are significant at 5% significance level. This is because, the differences in the mean values among the treatment groups (days after pesticide application) were greater than would be expected by chance; therefore there is a statistically significant difference ($P < 0.001$).

Physical removal of pesticide residue concentration from a crop is a way of reducing the concentration of the pesticide below acceptable levels. Pesticide residues concentration below acceptable levels implies that the crops are safer for consumption by humans and that there is no expected health implication associated with the consumption of the product. Pre-household treatment procedures are used commercially and in the home to reduce pesticide residues concentrations to levels below the MRL value. Pre-household treatment procedures used in this study are washing under running tap water (T_1), dipping in salt water and subsequent washing (T_2) and mild detergent washing (T_3).

Washing under running tap is prescribed for surface pesticide removal from vegetables. The effect of washing lettuce leaves under running tap for two minutes before cutting into pieces showed that washing is a means of reducing pesticide levels on lettuce. This is because removal of chlorpyrifos required a physical force which can be provided by the running tap water. As the running water flushes the leaves, surface pesticide are dislodged and washed away. The location and age of residues (Kumari, 2008) are factors that influence the dislodging of the residues. The extent of penetration into the leaves is highly dependent on the physicochemical properties of the compound.

Chlorpyrifos is a non-systemic insecticide and therefore would be located on the surface of the leaves after its application. The loosely bound surface residues of chlorpyrifos were therefore removed by washing under running tap water. However, 22.2% of the samples analyzed after household treatment procedure under running tap water had residue levels above the Maximum Residue Level (MRL) of 0.05 mg kg^{-1} (FAO/WHO, 2004) for chlorpyrifos on lettuce. This was observed on samples taken 1 hour after pesticide application. Household treatment procedure

under running tap water was less effective compared to other pre-household treatment procedures. This treatment caused a mean percentage reduction of 14.7% (Table 4.13) of chlorpyrifos levels.

From the results of ANOVA presented in appendix C, the values obtained after pre-household treatment procedure of washing under running tap water are significant at 5% significance level. The differences in the mean values among the treatment groups (days after pesticide application) are greater than would be expected by chance; there is a statistically significant difference ($P < 0.001$).

Results from this study show that, detergent is more effective in reducing chlorpyrifos levels on lettuce than salt solution. Dipping lettuce in 1% detergent (T_3) followed by thorough washing under tap water reduced the residues by a mean percentage of 54.4%, while salt water washing caused a mean percentage reduction of 33.8% (Table 4.13). This shows the effectiveness of detergent wash in chlorpyrifos removal. It was 3.7 times more efficient compared to tap water wash and 1.6 times more efficient compared to salt water washing. Moreover, the Maximum Residue Level (MRL) of 0.05 mg kg^{-1} (FAO/WHO, 2004) for lettuce was not exceeded in any of the samples analyzed after pre-household treatment procedure of mild detergent treatment.

According to Hui *et al.*, 2010, the rate of hydrolysis of chlorpyrifos in aqueous solution increases as pH increases and also the stability of chlorpyrifos decreases as the pH increases. Smith, 1968 also stated that, hydrolysis of chlorpyrifos occurs readily at $\text{pH} > 7$. Detergent which was used in T_3 caused an increase in the pH of aqueous solution. This resulted in destabilizing chlorpyrifos

residue levels on the lettuce resulting in higher removal of chlorpyrifos. For instance, the residue level detected 1 hour after pesticide application for the control samples was $0.059 \pm 0.008 \text{ mg kg}^{-1}$ but after dipping in mild detergent followed by thorough washing under tap water there was a reduction in the levels to $0.018 \pm 0.006 \text{ mg kg}^{-1}$.

The values obtained after T_2 is significant. The differences in the mean values among the treatment groups (days after pesticide application) are greater than would be expected by chance; there is a statistically significant difference ($P < 0.001$) (Appendix D). The values obtained after pre-household treatment procedure of dipping in mild detergent and washing afterwards are significant at 5% significance level. The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.023$) (Appendix E).



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CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Vegetable farmers in the study area apply insecticides on their crops to improve the yield of their crops and enhance profitability. Most farmers particularly those at Quarters Farms, at least spray their lettuce crops five (5) times in one growing season of the crop. The inappropriate application of pesticides by farmers leads to high levels of residues on crops and also contaminates the ecosystem. Farmers are prone to potential health hazards since they do not have protective clothings during pesticides application.

The detectable residue levels of chlorpyrifos were above the Maximum Residue Levels (MRLs) of 0.05 mg kg^{-1} , 1 hour after pesticide application. However, 1 day after pesticide application, there was dissipation of chlorpyrifos leading to a 17.9% reduction of chlorpyrifos levels on lettuce. The residue levels reduced further after 7 days with a mean percentage reduction of 91.1%. Despite the loss of most of the chlorpyrifos applied onto field-grown lettuce, residues were found in plant material 1 day after pesticide application, in concentrations sufficient to pose

a risk if lettuce consumed as fresh vegetables. Therefore, it is important to allow at least a 1-week (7 days) withholding period. After such a period, the residue concentration will have decreased to a level below the MRL.

A comparison of the overall effects of different pre-household treatment procedures indicated that levels of chlorpyrifos residues can be reduced significantly by washing under running tap water, salt water washing and by mild detergent washing. The effectiveness of different pre-household treatment procedures was observed in the order of $T_3 > T_2 > T_1$. Hence to reduce the risk associated with intake of chlorpyrifos through lettuce, mild detergent washing procedures should be followed before consumption. Nevertheless, lettuce should be rinsed thoroughly after the use of detergent, otherwise the detergent may be consumed together with the vegetables.

6.2 RECOMMENDATIONS

Ensuring food safety and to protect the public from consuming vegetables (lettuce) with high levels of pesticide residues remains a significant challenge in a developing country like Ghana where pesticides is used in vegetable farming.

It is therefore recommended that:

- Vegetable farmers who use pesticides should be educated on the need to ensure the withholding periods of pesticides and encouraged to adopt them to reduce residue levels on the vegetables.
- The public should be informed about the risks from contaminants in foodstuffs and the need to adopt improved pre-household treatment procedures during food preparation.

- Further studies should be carried out to determine whether the reduction of chlorpyrifos residue levels is through volatilization or photodegradation.

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APPENDICES

APPENDIX- A

QUESTIONNAIRE ON THE PERCEPTION, KNOWLEDGE AND ATTITUDES FOR SAMPLED FARMERS IN THE KUMASI METROPOLIS

PART 1- General Information

1. How many years have you had in the cultivation of vegetables?

2. Farm size you cultivate each cycle?

3. Educational level

No formal education [] Agric School/ Secondary []

Primary [] Tertiary []

4. Do you use pesticide in vegetable cultivation?

[YES] [NO]

PART2- Pesticide Choice

5. What type of pesticide do you use?

Insecticides	[]	Repellants	[]
Miticides	[]	Avicides	[]
Fungicides	[]	Herbicides	[]

6. Trade/brand name of the pesticide?

7. What criteria do you use in the selection of pesticide type for use?

Efficacy	[]	Availability	[]
Level of safety	[]	Cost	[]

Indicate if the choice is more than one or any other apart from the listed.

8. How do you obtain your information regarding the pesticide?

Label	[]	Advertisement	[]
Extension Agents	[]	Friends	[]

PART 3- Application Practices and Dose

9. Do you apply the pesticides in single or in combination?

[SINGLE] [COMBINATION]

10. If in COMBINATION, which brands are mostly used?

11. How do you estimate/measure the amount/volume of pesticide?

No idea	[]	15ml measuring cup	[]
Empty milk tin s/m	[]	Empty tomato tin s/m	[]
Experience	[]	Lid of pesticide container	[]

12. Do you have precise application doses for specific pests and crop stages?

[YES]

[NO]

13. If YES, what are the doses for specific pests and crop stages?

14. What is the time interval between the last time of pesticide application and harvest?

1 day []

2 days []

5 days []

7 days []

15. Do you own sprayer equipment?

[YES]

[NO]

16. What is the type of sprayer?

Hand pump (Knapsack sprayer) []

Motorized sprayer []

17. Do you wash sprayer after spraying?

[YES]

[NO]

18. How do you dispose off the wash water in the sprayer?

In irrigation canal []

On field []

In nearby stream []

PART 4- Protection During and After Pesticide Application

19. Do you protect yourself during pesticide application?

[YES]

[NO]

20. If YES, what personal protective equipments do you use?

Wellington boots []

Overalls []

Nose mask []

Handkerchiefs []

Eye goggles []

21. After pesticide application, how long does it take you to work on the beds (re-entry period)?

Less than 24h [] From 48 to 72h []

Less than 48h [] More than 72h []

22. How do you dispose off empty pesticide containers?

Rinsed [] Pile and sell []

Burnt on farm [] Bury in ground on farm []

Used as food and water receptacles [] Thrown away []



APPENDIX-B

ANOVA FOR CONTROL SAMPLES (T_0)

Normality Test: Passed ($P = 0.256$)

Equal Variance Test: Passed ($P = 0.491$)

Group Name	N	Missing	Mean	Std Dev	SEM
0 day	3	0	0.0563	0.00231	0.00133
1 day	3	0	0.0463	0.0107	0.00617
7 days	3	0	0.00500	0.00265	0.00153

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.00444	0.00222	52.621	<0.001
Residual	6	0.000253	0.0000422		
Total	8	0.00470			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P < 0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	$P < 0.050$
0 day vs. 7 days	0.0513	3	13.683	<0.001	Yes
0 day vs. 1 day	0.01000	2	2.666	0.109	No
1 day vs. 7 days	0.0413	2	11.018	<0.001	Yes

APPENDIX-C

ANOVA FOR TAP WATER WASHING TREATMENT (T_1) ON LETTUCE

Normality Test: Passed (P = 0.219)

Equal Variance Test: Passed (P = 0.450)

Group Name	N	Missing	Mean	Std Dev	SEM
0 day	3	0	0.0513	0.00208	0.00120
1 day	3	0	0.0393	0.00902	0.00521
7 days	3	0	0.00433	0.00208	0.00120

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.00358	0.00179	59.633	<0.001
Residual	6	0.000180	0.0000300		
Total	8	0.00376			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0 day vs. 7 days	0.0470	3	14.863	<0.001	Yes
0 day vs. 1 day	0.0120	2	3.795	0.037	Yes
1 day vs. 7 days	0.0350	2	11.068	<0.001	Yes

APPENDIX-D

ANOVA FOR SALT WATER WASHING TREATMENT (T₂) ON LETTUCE

Normality Test: Passed (P = 0.692)

Equal Variance Test: Passed (P = 0.498)

Group Name	N	Missing	Mean	Std Dev	SEM
0 day	3	0	0.0463	0.00643	0.00371
1 day	3	0	0.0260	0.00361	0.00208
7 days	3	0	0.00333	0.00115	0.000667

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.00278	0.00139	74.808	<0.001
Residual	6	0.000111	0.0000186		
Total	8	0.00289			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0 day vs. 7 days	0.0430	3	17.290	<0.001	Yes
0 day vs. 1 day	0.0203	2	8.176	0.001	Yes
1 day vs. 7 days	0.0227	2	9.114	<0.001	Yes

APPENDIX-E

ANOVA FOR MILD DETERGENT WASHING TREATMENT (T₃) ON LETTUCE

Normality Test: Passed (P = 0.189)

Equal Variance Test: Passed (P = 0.450)

Group Name	N	Missing	Mean	Std Dev	SEM
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0 day	3	0	0.0380	0.0173	0.01000
1 day	3	0	0.0187	0.00850	0.00491
7 days	3	0	0.00267	0.00153	0.000882

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.00188	0.000939	7.520	0.023
Residual	6	0.000749	0.000125		
Total	8	0.00263			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.023$).

Power of performed test with $\alpha = 0.050$: 0.699

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	$P < 0.050$
0 day vs. 7 days	0.0353	3	5.476	0.019	Yes
0 day vs. 1 day	0.0193	2	2.996	0.079	No
1 day vs. 7 days	0.0160	2	2.480	0.130	No

APPENDIX-F: LIST OF PLATES



Plate I. Lettuce plant



Plate II. An overview of the Quarters Farm Site



Plate III. A farmer applying pesticide



Plate IV. Soxhlet apparatus in use (for extraction)



Plate V. Rotary evaporator in use (to concentrate extract)



APPENDIX-G: GAS CHROMATOGRAPH OF CHLORPYRIFOS

