# KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

## COLLEGE OF SCIENCE



### DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

# LAMBDA CYHALOTHRIN RESIDUE IN ORANGES (*Citrus sinensis*) FROM BOAMADUMASI IN EJISU - JUABEN MUNICIPALITY OF THE ASHANTI REGION.

**BY**

**OWUSU KOFI ADJAPONG**

**NOVEMBER, 2014.**

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**A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, KNUST, KUMASI IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF SCIENCE DEGREE IN ENVIRONMENTAL SCIENCE.**

**BY**

**OWUSU KOFI ADJAPONG**

**NOVEMBER, 2014.**

## DECLARATION

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



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REV. S. K. AKYEAMPONG …….. ……..
HEAD SIGNATURE DATE

## DEDICATION

This is dedicated to my lovely wife Mrs. Gladys Adjapong and my children, Edwin Osei Adjapong and Jessica Nana Akoma Adjapong.

**ACKNOWLEDGEMENT**

I thank the Almighty God for his guidance throughout the period that I carried out this project work.

I am most grateful to my supervisors, Dr. J. Issifu Adam and Dr. Ray Bright Voegborlo for their immense support and constructive criticism that enabled me to complete this work successfully.

My sincere thanks also go to my parents, brothers and sisters for their prayers and encouragement.

Finally, my sincere gratitude goes to Mr. Tordzagla Nestor, Mr. Anthony Asamoah, Mr. Darkwa Maxwell Coffie and Mr Isaac Boafoh for their support and advice.

## ABSTRACT

Residues resulting from the use of pesticides on fruits are a major concern in many countries. However, the hazards to the health of humans can be minimized to a great extent if these residues are kept below their prescribed safety level. Thirty (30) orange farmers were interviewed on the types of insecticides they use. Five insecticides were found to be commonly used on oranges by the respondent farmers with Lambda cyhalothrin being the most commonly used followed by Dimethoate. In this study, levels of Lambda cyhalothrin insecticide residue in oranges from farms at Boamadumasi were investigated to establish the safety of the fruits for consumption based on the WHO maximum residue limit of 0.2 mg/kg for citrus fruits. The insecticide residues in samples were extracted and cleaned up from the blended epicarps and mesocarps with ethyl acetate and florisil respectively. The extracts were cleaned up (purified) by using florisil. 60% of acetonitrile, 20% methanol and 20% water mixture was used as the mobile phase for the HPLC analysis. A flow rate of 1.0 ml/min with UV detection set at 254 nm was employed at room temperature. Levels of Lambda cyhalothrin were declined considerably from day 0 (1hr) to day 21 after the insecticide application. Out of 24 samples of oranges analysed, 16 representing 66.67 % had some levels of lambda cyhalothrin although below W.H.O Maximum Residue Limit of 0.2 mg/kg. The residue levels detected in the oranges ranged from 0.005 mg/kg to 0.191 mg/kg.

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# CHAPTER ONE

## INTRODUCTION

### 1.1 Background

Food safety is a topic of great interest globally (Becky and André, 2009). With recent contamination issues in a wide range of commodities, ensuring the quality of our food supply is becoming increasingly important. The presence of pesticide residues in food, wildlife and the environment is of growing concern (Becky and André, 2009). Oranges are nutritious and medicinal fruits. They are eaten to allay fever and catarrh. The roasted pulp is prepared as a poultice for skin diseases. The fresh peel is rubbed on acne. In the mid-1950s, the health benefits of eating peeled, whole oranges were much publicized because of its protopectin, bioflavonoids and inositol (related to vitamin B). The orange contains a significant amount of the vitamin-like glucoside, hesperidin, which is used for the treatment of capillary disease. Oranges and other citrus fruits are attacked by pests including citrus rust mites, mealy bugs, aphids etc (Morton, 1987). To ensure that oranges are made available to humans, these pests are often managed using pesticides **(**Better Health Channel Fact Sheet, 1999).

### 1.2 Problem statement

The use of surface applications of insecticide remains one of the most cost-effective and versatile means of controlling insect pests in the environment (Jutsum *et al*., 1984). Gunther and Jeppson (1960), maintain that, there can be no doubt of the justifiable and understandable anxiety felt by individuals and by government agencies about possible risks to our health from the prolonged ingestion of even small amounts of these chemicals added to foods as pest-control agents.

According to the World Health Organization (WHO, 1992), approximately three million people are intoxicated each year as a result of the use of pesticides. In addition to the toxicity to humans, the presence of these products in the environment poses a risk to water quality and the ecosystem (Veiga *et al*., 2006). A higher proportion of pesticide poisonings and deaths occur in developing countries where there are inadequate occupational safety standards, protective clothing, washing facilities, insufficient enforcement, illiteracy and insufficient knowledge of pesticide hazards (Pimentel and Greiner 1996).



Despite the importance of oranges in the provision of various nutrients, the plant is susceptible to attack by pests which cause considerable damage to the fruits. To manage these pests, the plants are sprayed with various types of pesticides. Lambda super which contains Lambda cyhalothrin as the active ingredient is one of the insecticides mostly used by farmers to manage pests on their orange farms at Boamadumasi in the Ejisu - Juaben Municipality in the Ashanti Region of Ghana. At Boamadumasi, farmers use the insecticides frequently to obtain higher yield and to manage insect pests. The overdose of insecticides applied by farmers makes the residue a problem, which might pollute our food and be harmful to our health. What is most alarming is that pesticide use is very indiscriminate in the area. There are areas where insecticides are used in excessive quantities. Such situations make monitoring and assessment of insecticides contamination very difficult.

Lambda cyhalothrin is a synthetic pyrethroid insecticide and acaricide used to control a wide range of pests in a variety of applications including control of insects and acaricides (Royal Society of Chemistry, 1991). Lambda cyhalothrin is used to control a wide spectrum of insect

pests, e.g. aphids, Colorado beetles, thrips, Lepidoptera larvae, coleopteran larvae and adults, etc., in cereals, hops, ornamentals, potatoes, vegetables, cotton, and other crops. It provides good control of insect-borne plant viruses **(**Environmental Canada Review, 1989). Lambda cyhalothrin products come in various forms including powders, pellets, liquids, small capsules, and ear tags containing the chemical.

However, residues are retained in the oranges which can be harmful to humans if taken over a long period of time. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence (Cox, 2002).

Very little studies have been carried out on pesticide residues in citrus in Ghana. No studies on Lambda cyhalothrin residue in oranges have been undertaken in the Ejisu-Juaben Municipality and the use of the Lambda cyhalothrin is on the rise in the Municipality, according to current information.

## 1.3 Research Objectives

### 1.3.1. Main Objective

- To determine Lambda cyhalothrin residue levels in oranges from farms at Boamadumasi in the Ejisu- Juaben Municipality.

### 1.3.2. The Specific objectives were to:

- Interview farmers to find out other insecticides liked by orange farmers in the Ejisu - Juaben Municipality**.**
- Analyze Lambda cyhalothrin insecticide residue levels in the epicarp of oranges from farms at Boamadumasi in the Ejisu - Juaben Municipality.

- Analyze Lambda cyhalothrin residue levels in the mesocarp of oranges from farms at Boamadumasi in the Ejisu - Juaben Municipality.

# CHAPTER TWO

## LITERATURE REVIEW

### 2.1. Orange

#### 2.1.1. General description

Oranges are round citrus fruits with fine-textured skins that are, of course, orange in colour just like their pulpy flesh; the skin can vary in thickness from very thin to very thick. Oranges usually range from approximately two to three inches in diameter (Cho *et al.*, 2004).

According to Nicolosi *et al.* (2000), orange is a small flowering tree growing to about 10 m tall with evergreen leaves, which are arranged alternately, of ovate shape with crenulated margins and 4–10 cm long. The orange fruit is a hesperidium (a fruit with rind), a type of berry (Herbst, 2001).

Orange trees are widely cultivated in tropical and subtropical climates for the delicious sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice, and also for the fragrant peel (Katz and Weaver, 2003). Dotted with minute glands containing an essential oil, the outer rind (epicarp) is orange or yellow when ripe; the inner rind (mesocarp) is white, spongy and non-aromatic. The pulp (mesocarp), yellow, orange or more or less red, consists of tightly packed membranous juice sacs enclosed in 10 to 14 wedge-shaped compartments which are readily separated as individual segments (Morton, 1987).

Citrus fruits are equally valuable among populations who need to overcome and prevent micronutrient deficiencies as well as those concerned with problems of over nutrition, obesity and

diet-related chronic diseases. For example, citrus is an ideal component of low-fat, sodium-restricted diets ((Economos and Clay, 1999).

Sweet orange, *Citrus sinensis* (L. Osbeck) is the most cultivated species of citrus in the world. It requires a dormant period or a drop in night temperature to colour its fruits when grown on tropical plains. Oranges are classified into two general categories; sweet and bitter-with the former being the type most commonly consumed. Popular varieties of the sweet orange (*Citrus sinensis*) include Valencia, Navel and Jaffa oranges, as well as blood orange, a hybrid species (Cho *et al.*, 2004).

Valencia is a late season orange variety. This fruit is sweet, juicy and has a golden hue to its flesh. They come in medium to large sizes and have thin skin (Maurer and Bradley, 1998). In the warmer months, the skin is tinged with shades of green, a natural process called regreening (Rajeev, 2011). It is smaller than the ' Navel' orange, with a thinner and tighter rind. It is far juicier and richer in flavor (Morton, 1987).

Navel is a type of orange formed due to a single mutation. When peeled, an undeveloped conjoined twin- like fruit is found at the base of the orange. Since the base of the fruit resembles a human navel, it is called Navel orange. This is a seedless variety, propagated through cuttings. Its flesh is very juicy and sweet (Rajeev, 2011).

The epicarp of Blood orange fruit is rose-tinted in color and it has a pink to dark reddish flesh, depending on its variety. Blood oranges are smaller in sizes, more aromatic in flavour and have red hues running through their flesh (Cho *et al.*, 2004). The season for this fruit is December to

July. The Blood orange comes in several varieties viz. Tarocco, Sanguinello and Moro (Rajeev, 2011).

Jaffa oranges have thick skin. Their flesh is sweet and juicy, with few seeds. They are oval in shape and have a strong aroma (Rajeev, 2011).

Citrus is most commonly thought of as a good source of vitamin C. However, like most other whole foods, citrus fruits also contain an impressive list of other essential nutrients, including both glycaemic and non-glycaemic carbohydrate (sugars and fibre), potassium, folate, calcium, thiamin, niacin, vitamin $B_6$, phosphorus, magnesium, copper, riboflavin, pantothenic acid and a variety of phytochemicals (monoterpenes, limonoids, flavanoids, carotenoids and hydroxycinnamic acid). In addition, citrus contains no fat or sodium and, being a plant food, no cholesterol (Whitney and Rolfes, 1999).

### 2.1.2. History of Oranges

Sweet orange does not occur in the wild. It is believed to have been first cultivated in southern China, northeastern India, or perhaps southeastern Asia, formerly Indochina (Morton, 1987). It is the most commonly grown tree fruit in the world (Morton, 1987). Sweet oranges were introduced into Europe around the 15th century by various groups including the Moors, and the Portuguese as well as the Italian traders and explorers who found them on their voyages to Asia and the Middle East (Cho *et al.*, 2004). Brazil is the largest orange producing nation in the world, and production is located primarily in the state of São Paulo, which accounts for approximately 80% of Brazil's production and 53% of total global production (GAIN, 2010).

### 2.1.3 Citrus Production in Ghana

Citrus is one of the most important fruit crops grown by both large and small scale farmers in Ghana. The crop is grown mainly in the Central, Eastern and Ashanti Regions of Ghana, but thrives well in other parts of the country with similar favourable climatic factors (Asare-Bediako *et al.,* 2013). It is the most important horticultural crops in Ghana with a production area of around 13,000 hectares (Sakyi, 2010). Cultivation of citrus is a source of livelihood to many people in the rural areas as well as those involved in retailing the fruits in urban centres (Asare-Bediako *et al.,* 2013). Citrus varieties grown in Ghana includes Sweet orange (*Citrus sinensis)* and Tangerine *Citrus reticulata* (Blanco). Other available varieties are Grape fruits (*Vitis vinifera*), Lemons (*Citrus limon*), Lime (*Citrus aurantifolia*), Tangors, Tangelos and Ortanique (AILAP, 2006).

Based on maturity times, we have the following orange varieties in Ghana: Early maturing (August – October) Ovaletto, Skkan; Mid-season (October – January) Obuasi, Mediterranean sweet and Red Blood; Late maturing (March – April) Late Valencia, Olinda and Frost Valencia (AILAP, 2006). Blood oranges and Valencia oranges are the predominant varieties found in Boamadumasi in the Ejisu - Juaben Municipality. The Blood and Valencia oranges are popularly known as Bar red and Water neck respectively by the farmers in this locality.

### 2.1.4. Properties of Orange fruits (*Citrus sinensis*)

The edible portion of a sweet orange fruit is about 40-50 % of the whole fruit. It contains 80-90 g water; 0.7-1.3 g protein; 0.1- 0.3 g fat; 12.0 – 12.7 g carbohydrates (sugars); 0.5 g fiber; 0.5 – 0.7 g ash; 200 IU vitamin A; 45 - 61 mg ascorbic acid; 0.5 – 2.0 citric acid. The energy value is

about 200 kJ/100 g. The glucoside hesperidin occurs in significant quantities with part of vitamin P (citrin) which activates vitamin C and has curative action on blood vessels (Verheij and Coronel, 1991).

### 2.1.5. Importance of Oranges

**Compounds in Orange peel may lower cholesterol as effectively as statin drugs**

A class of compounds found in citrus fruit peels called polymethoxylated flavones (PMFs) have the potential to lower cholesterol more effectively than some prescription drugs, and without side effects (Kurowska and Manthey, 2004).

**Long-acting liminoids in citrus add to their ability to promote optimal health**

In citrus fruits, limonene is present in the form of limonene glucoside, in which limonene is attached to a sugar (glucose) molecule. The bodies of humans easily digest this compound, cleaving off the sugar and releasing limonene (Galati *et al.,* 1994). Citrus fruit like oranges, grapefruit and lemons are high in citric acid (Feinberg, 1973). Orange is greatly enjoyed due to it nutritional value as it provides vitamin C for the repair of worn out tissues. Consuming citrus fruits rich in vitamin C can help prevent anaemia and its devastating consequences (Economos and Clay, 1999). Older people with greater intake of fruits and vegetables, and the corresponding nutrients vitamin C and folate, have been shown to perform better on cognitive tests (Ortega *et al.,* 1997). Potassium is an essential mineral that works to maintain the body's water and acid balance. One medium-sized orange and one 225 ml glass of orange juice provide approximately 235 mg and 500 mg of potassium, respectively (Whitney and Rolfes, 1999). According to

Ferguson and Spann (2002), citrus flavonoids have potential antioxidant (prevents aging), anti-cancer, antiviral, anti-inflammatory activities, effects on capillarity, and cholesterol-lowering ability. Oranges are rich in a compound called citrus limonoids, which have been proven to help fight a number of varieties of cancer, including that of the skin, lung, breast, stomach and colon. Limonoids inhibit the development of breast cancer cells as well as reducing cholesterol (Ferguson and Spann, 2002).



A low dietary intake of folate contributes to the decrease of plasma folate and the raising of plasma homocysteine levels (Bloom, 1998; Tucker *et al.*, 1996). Homocysteine is a toxic agent for the vascular wall and when plasma levels rise above normal, there is an increased risk of cardiovascular disease. An inverse dose-response relationship has been identified for fruit and vegetable intake and plasma homocysteine levels. Frequent consumption of folate-rich foods, such as oranges and orange juice, tends to increase plasma folate levels and, thus, lower homocysteine levels.

**Protection against cardiovascular disease**

A diet that features citrus fruits also offers protection against cardiovascular diseases due to citrus fruits' folate, which is necessary for lowering levels of the cardiovascular risk factor, homocysteine; their potassium, which helps lower blood pressure, protecting against stroke and cardiac arrhythmias; and the vitamin C, carotenoids and flavonoids found in citrus fruits, all of which have been identified as having protective cardiovascular effects (Kurl *et al.*, 2002, Economos and Clay, 1999). Phytonutrients, specifically, the class of polyphenols, are high in citrus with oranges containing 84 mg Gallic Acid equivalents/100mg. The polyphenols so

abundant in oranges have been shown to have a wide range of antioxidant, anti-viral, anti-allergenic, anti-inflammatory, anti-proliferative and anti-carcinogenic effects (Rapisarda *et al*., 1999).

One major contributor in the development of heart disease appears to be a high level of oxidized low-density lipoprotein (LDL), the so-called bad cholesterol. Significantly, a recent study has shown that high intake of vitamin C (500 mg/day) obtained from the juice of freshly squeezed oranges, prevented a rise in the levels of oxidized LDL, even in the presence of a high-saturated fat diet (Harats *et al.*, 1998).

**Protection against Rheumatoid Arthritis**

Enjoying a daily glass of freshly squeezed orange juice can significantly lower your risk of developing rheumatoid arthritis, a chronic disease of joints that causes stiffness, swelling, weakness, loss of mobility, that leads to damage and eventual destruction of the joints (Pattison *et al.*, 2004).

**A very good source of fibre**

The fibre in oranges may be helpful for reducing the uncomfortable constipation or diarrhoea in those suffering from irritable bowel syndrome (Cho *et al.*, 2004).The fibre in oranges can grab cancer-causing chemicals and keep them away from cells of the colon, providing yet another line of protection from colon cancer.

According to Falsetto (2008), Citrus fruits are rich in Vitamin C or ascorbic acid and folic acid, as well as a good source of fibre and fluid which help to prevent constipation. Fibre ca n also

keep blood sugar levels under control, which explains why oranges can be a very healthy snack for people with diabetes (Cho *et al.,* 2004). In addition, the natural fruit sugar in oranges, fructose help to keep blood sugar levels from rising too high after eating. They are low in calories, and therefore help to control weight (Pattison *et al.*, 2004).

**Protect respiratory health**

Consuming foods rich in beta-cryptoxanthin, like orange-red carotenoid found in highest amounts in oranges, corn, pumpkin, papaya, red bell peppers, tangerines, and peaches, may significantly lower one's risk of developing lung cancer (Yuan *et al.*, 2003).

**Reducing birth defects**

During the first stage of pregnancy, adequate folate intake is critical for reducing the risk of severe birth defects, namely spina bifida and anencephaly. Public health recommendations in the United States includes the consumption of 400 mg of folate per day for women of child-bearing age (Centers for Disease Control and Prevention, 1992, Economos and Clay, 1999).

**Foreign exchange earnings**

Citrus is a source of foreign exchange earnings for Ghana through the export of the fresh fruit to neighbouring countries such as La Côte d'Ivoire, Burkina Faso and Togo. For instance, in the year 2010, 10,729 metric tonnes of fresh oranges were exported, generating foreign exchange of US$ 654,000 to the country (*MoFA,* 2011). Ghana produces around 250,000 tonnes of citrus annually representing 40% of fruit production and 36% of citrus exports. The Ghana citrus

industry is the largest fresh fruit exporter in Ghana, worth in excess of GH¢200 million annually (Sakyi, 2010).

### 2.1.6. Pests of Citrus

Mealy bugs form white masses underneath and between fruits in the early stages of development and may cause secretion of honeydew which forms a nutrient source for the fungal association termed sooty mould (Morton, 1987). The soft brown scale insect *Coccus hesperdium* (Linnaeus), occur on the fruits, leaves, and branches or trunks and may cause the die-back of twigs and premature fall of leaves and fruit (Yayock *et al.,* 1988).

The citrus blackfly, *Aleurocanthus woglumni (*Ashby), deposits eggs in spiral formations on the abaxial side of the leaves. Aphids (plant lice) cause leaves to curl and become crinkled. The orange dog is a large brown and white caterpillar, the larva of a black-and-yellow, swallowtailed butterfly which damages the trees in summer and autumn (Morton, 1987). The false codling moth *Argyroploce curvipes* (Meyr), causes serious losses in citrus plantations by piercing the fruits thereby causing them to rot and fall from the tree (Yayock *et al.,* 1988).

Fruit fly, *Ceratitis capitata* (Wiedemann), is the major insect pest of citrus in Ghana. The fly lays its eggs in the fruit leading to premature ripening and fruit drop (AILAP, 2006). Aphids can do serious damage, especially to young trees with new and tender foliage. The black citrus aphid, *Toxoptera citricidus* (Kirkaldy), damages nursery trees and transmits tristeza or dieback. Another aphid, *Toxoptera aurantii* (Fonscolombe), causes leaf curl. Mites also cause leathery leaves and distorted rinds of fruits (AILAP, 2006).

Other pests of citrus include citrus gall wasp, citrus leafminer, *Phyllocnistis citrella* (Stainton) common insect pests of nectarines, cottony cushion scale, *Icerya purchasi* (Maskell), crusader bug *Mictis profana* (Fabricius), fuller's rose weevil, *Naupactus godmanni* (Crotch), light brown apple moth, *Epiphyas postvittana* (Walker), mites, pezothrips, red scale, spined citrus bug, , *Biprorulus bibax* (Ivess), (Morton, 1987).

## 2.2. Lambda Cyhalothrin



### 2.2.1 General description

Lambda cyhalothrin is a synthetic pyrethroid consisting of two of the four enantiomeric forms of Cyhalothrin. Lambda cyhalothrin consists of the more active pair of enantiomers of cyhalothrin (WHO, 1990). Pyrethroids are synthetic chemical analogues of pyrethrins, which are naturally occurring insecticidal compounds produced in the flowers of chrysanthemums, *Chrysanthemum cinerariaefolium*, (Amweg and Weston, 2005; IPCS, 1990a).

Lambda cyhalothrin is a pyrethroid that acts predominantly on the central nervous system; high dosages have been found to cause tonic seizures in experimental animals. A high concentration in air may be irritant, and contact with the concentrated product may induce a temporary tingling sensation, particularly on the face. It may be hazardous if swallowed (WHO, 1997a).

### 2.2.2. Uses

Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, and homes and gardens (Amweg and Weston, 2005; Oros and Werner, 2005). Lambda cyhalothrin is used as a stomach and contact insecticide in agriculture; it is also

used in public and animal health (WHO, 1990). Lambda cyhalothrin is a synthetic pyrethroid most commonly used for pest control, especially mosquitoes (IPCS, 1990a). In addition to mosquitoes, it is effectively used to control: cockroaches, ticks, fleas, aphids, Colorado beetles, cutworms and butterfly larvae (EXTOXNET, 1996; IPCS, 1990a).

All pyrethroids are potent neurotoxicants that interfere with nerve cell function by interacting with voltage- regulated sodium channels as well as other ion channels, resulting in repetitive firing of neurons and eventually causing paralysis (Shafer and Meyer, 2004). Due to the lipophilic nature of pyrethroids, biological membranes and tissues readily take up pyrethroids (Oros and Werner, 2005).

In commercial applications, lambda cyhalothrin is used on food crops, non-food crops, in greenhouses, in and around hospitals, for cattle (in ear tags), and in termite treatments. Residential use can be both indoors and outdoors on homes, ornamental plants, and lawns (NPTN, 2008).

### 2.2.3. Identity of Lambda Cyhalothrin

Lambda cyhalothrin is a mixture of highly active isomers of cyhalothrin (NPTN, 2008). Technical grade lambda cyhalothrin is a very pale brown solid and contains more than 90% active material. The enantiomer ratio of the *(Z), (1R, 3R),* S-ester to the *(Z), (1S, 3S),* R-ester is 1:1. It is sparingly soluble in water but soluble in a range of organic solvents and has a low vapour pressure (IPCS, 1990a).

**Molecular formula**: $C_{23}H_{19}ClF_3NO_3$

**Trade names**: Lambda cyhalothrin: "Karate", "Matador", "Icon" and "Grenade"

**CAS chemical name**: *(R+S)*-alpha-cyano-3-(phenoxyphenyl) methyl- *(1S+1R)-cis*-3-(*z*-2-chloro-3, 3, 3,-trifluoroprop-1-enyl)-2, 2-dimethylcyclopropane- carboxylate.

**Chemical name**: alpha-cyano-3-phenoxybenzyl-3-(2-chloro- 3, 3, 3-trifluoroprop-1-enyl)-2, 2- dimethyl - cyclopropane-carboxylate.

**CAS registry number**: lambda cyhalothrin: 91465-08-6 (Kegley *et al.*, 2010; European Commission, 2001).

### 2.2.4. Chemical structure of Lambda Cyhalothrin

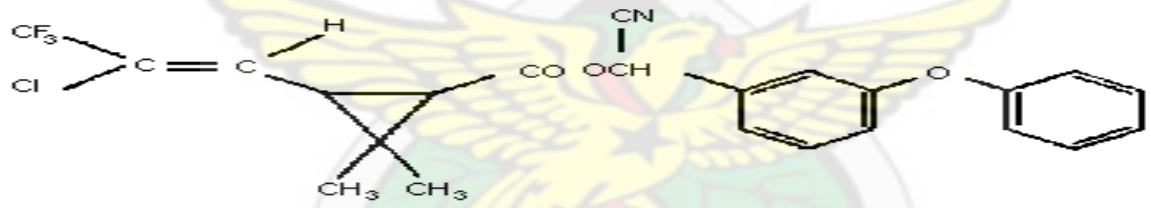


Fig. 1: Chemical structure of Lambda Cyhalothrin
Source: European Commission, 2001**.**

### 2.2.5. Physical properties of lambda Cyhalothrin

**Relative molecular mass**: 449.9

**Melting point (°C):** 47.5- 49.2

**Decomposition (°C):** > 275

**Water solubility**: $5 \times 10^{-3}$ mg/litre

**Solubility in organic solvents:** At 21 $^{o}$C: hexane, toluene, dichloromethane, methanol, acetone and ethyl acetate: > 500 g/l (Jackson, 1994).

**Partition Coefficient (octanol/water):** 10,000,000

**Adsorption Coefficient**: 180,000

**Density:** 1.33 g/ml

**Vapour pressure** : (kPa at 20°C) $2 \times 10^{-10}$ (kPa at 80°C) $3 \times 10^{-6}$ (WHO, 1990; European commission, 2001).



### 2.2.6. Formulation

Lambda cyhalothrin is available in powders, pellets, and small capsules. It is also placed in ear tags for cattle (NPTN, 2008). It is formulated as 2.5%, 5.0%, 8.3%, and 12% emulsifiable concentrates and as a 0.8% ultra- low-volume (ULV) formulation (WHO, 1990).

### 2.2.7. Routes of exposure

The substance can be absorbed into the body by inhalation of fine dust and mist, and by ingestion (IPCS CEC, 1993). Pyrethroids usually enter the body when people eat foods contaminated by these chemicals. They may also enter the body when one breathes air that contains these compounds or when one gets them on the skin (ATSDR, 2003).

### 2.2.8. Degradation of lambda cyhalothrin

#### 2.2.8.1. Degradation in the soil

On soil surfaces and in aqueous solutions at pH 5, lambda-cyhalothrin is degraded in sunlight with a half-life of approximately 30 days. The main degradation products are 3-(2-chloro-3, 3, 3-

trifluoroprop1-enyl) - 2-dimethylcyclopropane carboxylic acid, the amide derivative of cyhalothrin, and phenoxybenzoic acid (WHO, 1990).

Degradation in soil occurs primarily through hydroxylation followed by cleavage of the ester linkage to give two main degradation products that are further degraded to carbon dioxide. The initial half-lives are in the range of 22-82 days (WHO, 1990).

Cyhalothrin and lambda-cyhalothrin are adsorbed on soil particles and are non-mobile in the environment.



#### 2.2.8.2. Degradation on plants

On plants, lambda-cyhalothrin degrades at a moderate rate (half-life up to 40 days) and the major constituent of the residue on plants is usually the parent compound. Lower levels of metabolites, resulting from a range of hydrolytic and oxidative reactions, are also found (WHO, 1990).

### 2.2.9. Effects of Lambda Cyhalothrin

#### 2.2.9.1. Effects on animals

Like many compounds of the pyrethroid family, the observed toxicity of lambda cyhalothrin may vary according to not only the concentration of the active ingredient but also according to the solvent vehicle (Meister, 1992).

The substance irritates the eyes, the skin and the respiratory tract. The substance may cause effects on the peripheral nervous system, resulting in convulsions, ataxia (IPCS CEC, 1993).

Cyhalothrin and lambda cyhalothrin have a high potential for bioaccumulation, with bioconcentration factors of 770 (estimated) and 1660-2240 (whole fish) respectively. Both

substances have high octanol water partition coefficients (logKow = 6.8 and 7.0). Lambda-cyhalothrin has adulticidal, ovicidal, and larvicidal activity (IPCS, 1990a). Under laboratory conditions of constant toxicant concentrations, cyhalothrin and lambda-cyhalothrin were highly toxic to fish and aquatic invertebrates. The 96-h $LC_{50}$s for fish ranged between 0.2 and 1.3 µg/litre; the 48-h $LC_{50}$s for aquatic invertebrates ranged between 0.008 and 0.4µg/litre (IPCS, 1990b). Like other pyrethroids, lambda cyhalothrin disrupts the nervous system in insects, causing paralysis and death (NPTN, 2008). It is highly toxic to bees, for which the oral $LD_{50}$ is 38 ng/bee, and the contact $LD_{50}$ is 909 ng/bee (EXTOXNET, 1996).

Lambda cyhalothrin has a very low toxicity in birds. The oral $LD_{50}$ in mallard duck is greater than 3,950 mg/kg (EXTOXNET, 1996).

Lambda cyhalothrin is highly toxic to bees, with a reported oral $LD_{50}$ of 38 ng/bee and reported contact $LD_{50}$ of 909 ng/bee (0.9 ug/bee).

Lambda cyhalothrin is very toxic to fish. Some studies have indicated that the chemical may accumulate in these organisms (NPTN, 2008).

**2.2.9.2. Effects on Humans**

Following substantial ingestion of lambda cyhalothrin, patients may fall into coma, convulsions, and severe muscle fasciculations, and may take several days and occasionally weeks to recover (RTI International, 2007). It is also possible that the impacts of normal residential exposure of pregnant women could include neurological effects on unborn foetuses, but further research is necessary to test this hypothesis (Berkowitz *et al*., 2003).

Human inhalation of toxic fumes in the event of a storehouse, fire is also an unavoidable risk, as open-burning of lambda cyhalothrin creates nitrogen oxides, hydrogen chloride, and hydrogen fluoride (WHO, 1997b). Lambda cyhalothrin can have corrosive effects on both the skin and eyes. Workers handling it reported facial tingling and burning, which lasted six hours to two days. Ingested lambda cyhalothrin is moderately toxic, although mammals are found to metabolize and excrete lambda cyhalothrin rapidly. The EPA identifies lambda cyhalothrin as a group D carcinogen, an undetermined human carcinogen (NPTN, 2008).

Exposure to lambda cyhalothrin may occur through inhalation, dermal absorption, or ingestion. Inhalation may cause burning sensations, convulsions, coughing, labored breathing, shortness of breath, and sore throat. Contact with the skin and eyes may cause redness and pain. Ingestion can cause abdominal pain and coughing (PANNA, 2010; He *et al.*, 1989; Kegley *et al*., 2010). Extreme levels of exposure can also cause seizures and coma (NPTN, 2008). The effects of respiratory diseases and skin disorders may intensify with exposure to lambda cyhalothrin (NLM, 2001).

### 2.2.10. Analytical Methods

The most widely adopted procedures for the determination of cyhalothrin residues in crops, animal tissues and products, soil, and other environmental samples are based on extraction of the residue with organic solvent and clean-up of the extract as necessary by solvent-solvent partition and adsorption column chromatography, followed by determination of the residue using gas chromatography (GC) with electron capture detection (GC/ECD) or high performance liquid chromatography (HPLC). The identity of residues can be confirmed by GC with mass selective

detection (GC-MSD) or by thin-layer chromatography (TLC) followed by GC/ECD (WHO,1990).

### 2.3. Chromatography

#### 2.3.1. General Description

Chromatography is a method for analyzing complex mixtures (such as ink) by separating them into the chemicals from which they are made (Mag, 2003). The technique depends on the principle of selective adsorption, a type of adhesion. Schoffstall *et al.* (2000), defines Chromatography as the separation of components of a mixture by differential adsorption between a stationary phase and a mobile phase.

Chromatography is used in many different industries and laboratories. Chemists use chromatography in laboratories to track the progress of a reaction. By looking at the sample spots on the chromatography plate, they can easily find out when the products start to form and when the reactants have been used up (i.e., when the reaction is complete). Chemists and biologists also use chromatography to identify the compounds present in a sample, such as plants **(**Clark, 2007). Chromatography is essential to the separation of pure substances from complex mixtures and is widely used in the analysis of foods, drugs, blood, petroleum products, and radioactive-fission products (Pascal *et al*., 2000).

According to Schoffstall *et al.* (2000), the chromatographic methods most frequently used by organic chemists are gas-liquid chromatography, column chromatography, thin-layer chromatography and high-performance liquid chromatography.

### 2.3.2. Gas Chromatography

Gas – liquid chromatography (GC) is used for separations of volatile or reasonably volatile organic liquids and solids. The stationary phase for GC is usually an organic polymer coated on the inside of a tube, such as a long capillary tube, and the mobile phase is an inert gas, such as helium (Schoffstall *et al.,* 2000). In gas chromatography (GC), the stationary phase is a high boiling liquid and the mobile phase is an inert gas. It can also be used to separate small amounts of material (Karasek and Raye, 1988).



### 2.3.3. Thin Layer Chromatography

Thin Layer Chromatography (TLC) is the separation of moderately volatile or nonvolatile substances based upon differential adsorption on an inert solid (stationary phase) immersed in an organic solvent or solvent mixture (mobile phase). The components are distributed between the stationary phase (usually silica gel or alumina) and the solvent depending upon the polarities of the compound and solvent (Schoffstall *et al.,* 2000). A particular advantage is that it allows the analysis of many samples simultaneously. In TLC, the different components of the sample are separated by their interaction with the stationary phase (bonded to the glass, aluminum, or plastic support) and the liquid mobile phase that moves along the stationary phase (Sherma, 1991; Poole, 1999).

### 2.3.4. High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a type of column chromatography designed to separate and classify components of a sample based on its interactions with a specific column (Schoffstall *et al.,* 2000). Schoffstall *et al.* (2000), indicated that HPLC is most

useful as a quantitative analytical method, much the same as Gas Chromatography (GC). The method resembles GC but instead of a carrier gas, a solvent is used as the mobile phase and the most common detector uses UV detection. HPLC is distinct because it utilizes a more pressurized environment to propel the liquid samples through the densely-packed column more efficiently (Lane, 2010).

According to Dong (2006), HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase (Dong, 2006).

The extent to which a component is retained in the column is determined by its partitioning between the liquid mobile phase and the stationary phase. In HPLC, this partitioning is affected by the relative solute/stationary phase and solute/mobile phase interactions. Thus, unlike GC, changes in mobile phase composition can have an enormous impact on your separation (Brown *et al.*, 1997). An HPLC method was developed for the analysis of lambda-cyhalothrin residue in drinking water (from polluted rivers) and *Brassica chinensis* (Linnaeus) leaves. The analysis was performed on a SB-C18 column using acetonitrile-water (80:20,V/V) as a mobile phase at the flow rate of 1.25 ml/min and detection wavelength was set as 230 nm (Zheng *et al.*, 2009). The recoveries for lambda-cyhalothrin in drinking water and ground *Brassica chinensis* leaves spiked at three levels were in the ranges of 97.93% - 103.02% and 91.4% - 113.9%, respectively (Zheng *et al.*, 2009).

Table 1 below shows the recommended FAO/WHO Maximum Residue Limit for Lambda Cyhalothrin in some fruits, vegetables, meat, liver and kidney of ruminants.

**Table 1: FAO/WHO Maximum Residue Limit for Lambda Cyhalothrin adopted in 2010**

| Commodity | MRL |
|---|---|
| Root and tuber vegetables | 0.01 mg/kg |
| Tree nuts | 0.01 mg/kg |
| Maize | 0.02mg/kg |
| Asparagus | 0.02mg/kg |
| Sugar cane | 0.05mg/kg |
| Triticale | 0.05mg/kg |
| Liver of cattle, goats, pigs & sheep | 0.05mg/kg |
| Rye | 0.05mg/kg |
| Pulses | 0.05mg/kg |
| Wheat | 0.05mg/kg |
| Fruiting vegetables, cucurbits | 0.05mg/kg |
| Oats | 0.05mg/kg |
| Wheat bran, Unprocessed | 0.1mg/kg |
| Mango | 0.2mg/kg |
| Legume vegetables | 0.2mg/kg |

| | |
|---|---|
| Berries and other small fruits | 0.2mg/kg |
| Kidney of cattle, goats, pigs and sheep | 0.2mg/kg |
| Milks | 0.2mg/kg |
| Plums (including prunes) | 0.2mg/kg |
| Oilseed | 0.2mg/kg |
| Citrus fruits | 0.2mg/kg |
| Pome fruits | 0.2mg/kg |
| Bulb vegetables | 0.2mg/kg |
| Cherries | 0.3mg/kg |
| Fruiting vegetables other than cucurbits | 0.3mg/kg |
| Cabbages | 0.3mg/kg |
| Dried grapes (currants, raisins and sultanas) | 0.3mg/kg |
| Nectarine | 0.5mg/kg |
| Barley | 0.5mg/kg |
| Apricot | 0.5mg/kg |
| Peach | 0.5mg/kg |
| Olives | 1mg/kg |
| Rice | 1mg/kg |
| Straw and fodder(dry) of cereal grains | 2mg/kg |
| Almond hulls | 2mg/kg |

| | |
|---|---|
| Meat (from mammals other than marine mammals) | 3mg/kg |
| Pepper | 3mg/kg |

FAO/WHO, 2010.

# CHAPTER THREE

## MATERIALS AND METHODS

### 3.1. Study Area

The study was conducted in two orange farms at Boamadumasi in the Ejisu- Juaben Municipality of the Ashanti Region of Ghana. The orange variety grown on farm 1 was Blood orange also known as Bar red by the local farmers. Valencia oranges, locally called Water neck by the farmers were grown on Farm 2. Figure 2 shows the map of Ejisu – Juaben Municipality. Boamadumasi is a small town which branches and shares its boundary with another town called Duampompo which is along the trunk road of Konongo - Ejisu. The town has a population of 1,315 people and the main occupation of the area is farming (Municipal Statistical Service, 2010). Most of them are into staple food production such as garden eggs, maize, cassava, plantain, cabbage and orange. The land is slightly sloppy with houses dispersed without a regular pattern.

Rainfall of Boamadumasi is weakly bi-modal, the major rainfall season occurs between March and September, peaking in June and August with an annual average of about 1300 mm (Meteorological Services, 2012). The area has a wet, semi-equatorial climate temperatures averaging 26 $^{o}$C. The dry season (November to March) is sharp and pronounced. Soils in Boamadumasi belong to the Bekwai, Nzema, Kokofu and Oda series, which is poorly drained and are described as forest oxysols because of their 'sharp' or acidic nature (Adu, 1992).

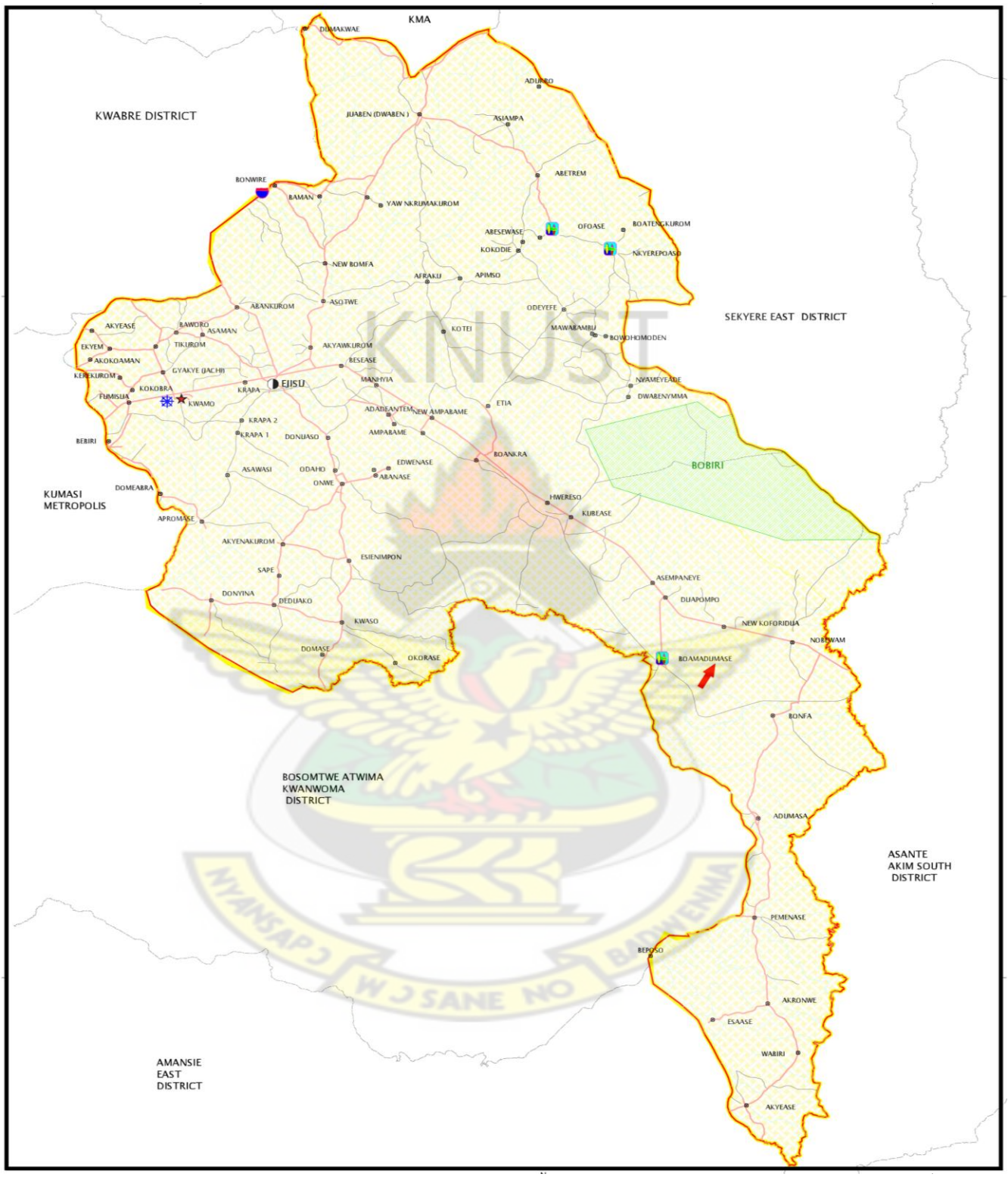


Fig. 2 Map of Ejisu- Juaben Municipality (Boamadumasi indicated with the red arrow)

### 3.2. Interviews conducted

Interviews were conducted on Orange farmers in the following towns in the Ejisu –Juaben Municipality; Abankro, Boamadumasi, Boankra, Duampompo, Ejisu, Juaben, Kubease and Kwamo. The focus of the interview was primarily on farmers who use insecticides on their crops. The purpose was to obtain information on insecticides used by farmers on their orange trees. In all, thirty (30) farmers were interviewed. Questions asked are presented in Appendix A.

### 3.3. Materials



#### 3.3.1 Instrumentation

Instrument: Varian Inc. Liquid Chromatograph with Varian Prostar 325 LC Detector and Varian Prostar 210/215/218/SD-1 pump, 705 SN 50µl Hamilton syringe, Reversed-phase Column (140mm × 4.6 mm internal diameter), Buchi Rotavapour R-114, Buchi Vacuum Pump V-700, Buchi water bath B - 480, 10 ml, 25 ml, 50 ml, 100 ml, 200 ml volumetric flask, Flat-bottom flasks, Round-bottom flasks, Beakers. Others were Electronic balance: Libror EB- 430Hw Shimazu capacity 430.000 g and Scout ProSPU401 capacity 400 g, 0.1ml, 1ml, 2ml, 5ml, 10ml Pipettes, 50 ml. 100 ml, 500ml Measuring Cylinders, Conical flasks, Funnels, Capillary tubes, High speed blender, Knife, Chopping board and Mechanical shaker.

#### 3.3.2 Reagents and Chemicals

Florisil, Ethyl acetate (BDH Laboratory Reagents), Methanol (Harris Reagent), Anhydrous Sodium sulphate (ALDRICH Chemicals), Lambda cyhalothrin. Others were Hexane (BDH Laboratory Reagents), Dichloromethane (BDH Laboratory Reagents), Acetonitrile (SIGMA ALDRICH), Distilled water.


**Plate 1. Lambda cyhalothrin used by farmers**

### 3.4 Methods

#### 3.4.1 Field Survey to Identify Insecticides used to Spray Oranges

The field survey included collection of information from farmers through interviews. Only farmers actively involved in orange farming were interviewed.

On the basis of answers obtained from the interviews, Boamadumasi in the Ejisu- Juaben Municipality was selected for the study. Thirty (30) orange farmers were interviewed to generate

information. The types of insecticides used, their level of education and ways of disposing used insecticide containers. Other questions asked included the spraying machine used and whether farmers use protective clothing while they handle and apply insecticide.

Before conducting an interview, the objective of the study was briefly explained to the respondents highlighting the need, importance and the possible outcomes of the study. During the process of interviewing, a friendly environment was maintained so that correct and reliable information could be obtained.

### 3.4.2 Sampling of Oranges for Residues Analysis

During the field survey, fresh oranges were collected from farmers' fields to assess the residue levels of insecticides on the crops. A total of 60 oranges were collected from two farms for the study. Thirty fruits were collected from each farm and these were taken from high and low areas, those exposed and those sheltered by foliage. The fruits were transported to the laboratory in a box immediately after collection and extracted with ethyl acetate and anhydrous sodium sulphate and the extracts were stored below a temperature of 0 $^{0}$C until analysis to prevent spoilage.

### 3.4.3 Extraction of Insecticide Residues from Oranges

The oranges were sampled on 0 (1 hour), 1, 3, 7, 14 and 21 days after the last application of the insecticide. Orange fruits were collected into a paper box and transferred to the Department of Chemistry laboratory, Kwame Nkrumah University of Science and Technology. The epicarp of the fruits were peeled and separated from the mesocarp. Each portion of the epicarp or mesocarp was chopped with a sharp knife and mixed thoroughly. The chopped sample was transferred into

a high-speed blender; and was thoroughly blended to obtain a homogeneous sample. One hundred grams (100 g) of the sample was weighed into a 250 ml flat-bottom flask for extraction, 150 ml ethyl acetate and 50 g anhydrous sodium sulphate were added to the content of the flask and shaken for 1hour at 60 cycles/min in a horizontal shaker and then filtered to get the filterate. The resulting extract was evaporated to near dryness using a rotary evaporator at 40 ºC and poured into an evaporating dish. The mobile phase was used to dissolve the concentrated extract that remained in the evaporating dish and then analyzed using HPLC technique.



### 3.4.4 HPLC conditions and systems observed

Mobile Phase: A mixture of 60% Acetonitrile, 20% Methanol and 20% Water

Wavelength of detection = 254 nm

Flow rate =1.0 ml/min

Injection volume = 50 µl

Column: Reversed-phase ($C_{18}$)

Temperature: ambient temperature

Sensitivity: 0.500

### 3.4.5 Preparation of Stock Solution

A quantity of 0.1 g of the pure lambda cyhalothrin was weighed in a beaker using Electronic balance: Libror EB- 430Hw Shamzu capacity 430.000 g. Some of the mobile phase was then added to the content in the beaker and the solution was well shaken to ensure complete dissolution of the pure lambda cyhalothrin. The content was then transferred into 100 ml

volumetric flask using funnel. The beaker was rinsed several times with the mobile phase to ensure quantitative transfer of the lambda cyhalothrin and more of the mobile phase was added until it was up to the 100 ml mark and then stoppered and labeled. This solution constitutes a concentration of 1.0 mg/ml.



#### 3.4.5.1 HPLC Qualitative Determination of Pure Lambda cyhalothrin

A concentration of 0.04 mg/ml of the pure lambda cyhalothrin solution was prepared and injected into the column and its retention time was recorded. The wavelength was set at 254 nm.

#### 3.4.5.2 Calibration Curve for Pure Lambda cyhalothrin

Concentrations of 0.04 mg/ml, 0.12 mg/ml, 0.20 mg/ml, 0.28 mg/ml, 0.36 mg/ml, 0.44 mg/ml were prepared from the pure Lambda cyhalothrin stock solution 1.0 mg/ml by pipetting 1.0 ml, 3.0 ml, 5.0 ml, 7.0 ml, 9.0 ml and 11.0 ml respectively, with each diluted to 25.0 ml. The resulting solutions were filtered and some of the filtrates were taken from each and were injected into the column one after the other and their retention times recorded. The Lambda cyhalothrin extract from the sample was analysed at wavelength of 254 nm.

#### 3.4.5.3 HPLC Analysis of Extracts

HPLC analysis was performed using Varian Prostar 210/215/218/SD-1 pump, syringe loading sample injector fitted with external 50 µl loop, and Varian Prostar 325 LC detector. The stationary phase used was HPLC column of length 140 mm - 4.6 mm (internal diameter). A mixture of 60 % acetonitrile, 20 % methanol and 20 % water was used as a mobile phase. Prior

to use, the mobile phase as well as the extract was filtered. The mobile phase was first loaded into sample injector, and then later the extracts were also loaded. The response was reproducible and co-extracts in the samples showed less interfering peaks under UV detection.

### 3.4.6. Validation of Analytical Method

#### 3.4.6.1 Repeatability (Within-run Precision)

Concentrations of 0.20 mg/ml, 0.36 mg/ml and 0.44 mg/ml of pure lambda cyhalothrin were prepared. Each solution was filtered to remove particles from it, and samples from these solutions were successively run five times in the liquid Chromatograph. This was done on the same day and under the same laboratory conditions. The average peak area, standard deviation and relative standard deviation were determined statistically for each concentration taken.

#### 3.4.6.2 Reproducibility (Between-run Precision)

The analysis was carried out on three different days by three different analysts and the results analysed statistically for mean ($\bar{x}$) standard deviation (SD) and relative standard deviation (RSD).

A concentration of 0.04 mg/ml of pure lambda cyhalothrin solution was accurately prepared. The solution was filtered to remove any particles before the analyte was introduced onto the column. A sample from the solution was successively run five times a day after stabilizing the chromatographic system and this was done for three days. The various solutes were determined by their retention times and the average peak areas were calculated for each set that was run per day.

### 3.4.6.3 Precision

This was assessed by replicate injections as performed in both repeatability and reproducibility of the method where standard deviation and relative standard deviation were calculated.

### 3.4.6.4 Linearity

Concentrations of 0.04 mg/ml, 0.12 mg/ml, 0.20 mg/ml, 0.28 mg/ml, 0.36 mg/ml, 0.44 mg/ml were prepared from the pure Lambda cyhalothrin stock solution of 0.001 g/ml by pipetting 1.0 ml, 3.0 ml, 5.0 ml, 7.0 ml, 9.0 ml and 11.0 ml respectively, with each diluted to 25.0 ml. The resulting solutions were filtered and some of the filtrates were taken from each and were injected into the column one after another and their retention times recorded. The Lambda cyhalothrin sample was analysed at a wavelength of 254 nm.

All determinations were done in triplicate and the average peak area for each of the concentration was calculated and tabulated. A graph of average peak area on the vertical axis was plotted against concentration on the horizontal axis and the regression coefficient ($r^2$) determined. The aim of checking linearity is to derive a direct proportionality between the detector signal and the concentration of a substance in the sample over a certain range.

### 3.4.6.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection (LOD) is the smallest concentration of a substance in a sample (matrix) that can be detected but not necessarily quantitated. Limit of quantitation (LOQ) on the other hand is the smallest amount of a substance in a sample (matrix) that can be quantified as an exact value with acceptable precision and accuracy. The two parameters were determined by the use of the

standard deviation of the calibration line by determining the slope and standard deviation (SD) of the response.

Thus LOD = (3.3σ) / S

LOQ = (10σ) / S

Where σ = standard deviation of the response. S = Slope of the calibration graph.

# CHAPTER FOUR

## RESULTS

### 4.1 Survey results

#### 4.1.1 Towns visited and the insecticides used

**Table 2: Insecticides used for managing insect pest on Orange Trees in indicated towns in the Ejisu – Juaben Municipality.**

| Towns visited | Insecticides | | | | | Total |
|---|---|---|---|---|---|---|
| | Lambda Cyhalothrin | Chlorpyrifos | Dimethoate | Imidacloprid | Cypermethrin | |
| Abankro | 1 | 1 | 1 | 0 | 0 | 3 |
| Boamadumasi | 4 | 1 | 1 | 1 | 0 | 7 |
| Boankra | 2 | 0 | 0 | 1 | 0 | 3 |
| Duampompo | 2 | 1 | 1 | 0 | 0 | 4 |
| Ejisu | 1 | 0 | 2 | 1 | 1 | 5 |
| Juaben | 2 | 1 | 1 | 0 | 0 | 4 |
| Kubease | 2 | 0 | 0 | 0 | 0 | 2 |
| Kwamo | 2 | 0 | 0 | 0 | 0 | 2 |
| Total | 16 | 4 | 6 | 3 | 1 | 30 |

Towns visited and insecticides used by the farmers are shown in Table 2 and in appendices A and B. It was found out that all farmers interviewed in all the towns visited used various kinds of

insecticides for spraying their orange trees. At least one farmer from each town used Lambda cyhalothrin. The most commonly used insecticide for spraying oranges in the Ejisu – Juaben Municipality was Lambda cyhalothrin representing 53.33% of respondents. Thus out of 30 orange farmers that were interviewed, 16 farmers mentioned lambda cyhalothrin being the most preferred insecticide, 6 mentioned dimethoate as insecticide used, 4 used chlorpyrifos, 3 used imidacloprid and 1 used cypermethrin.



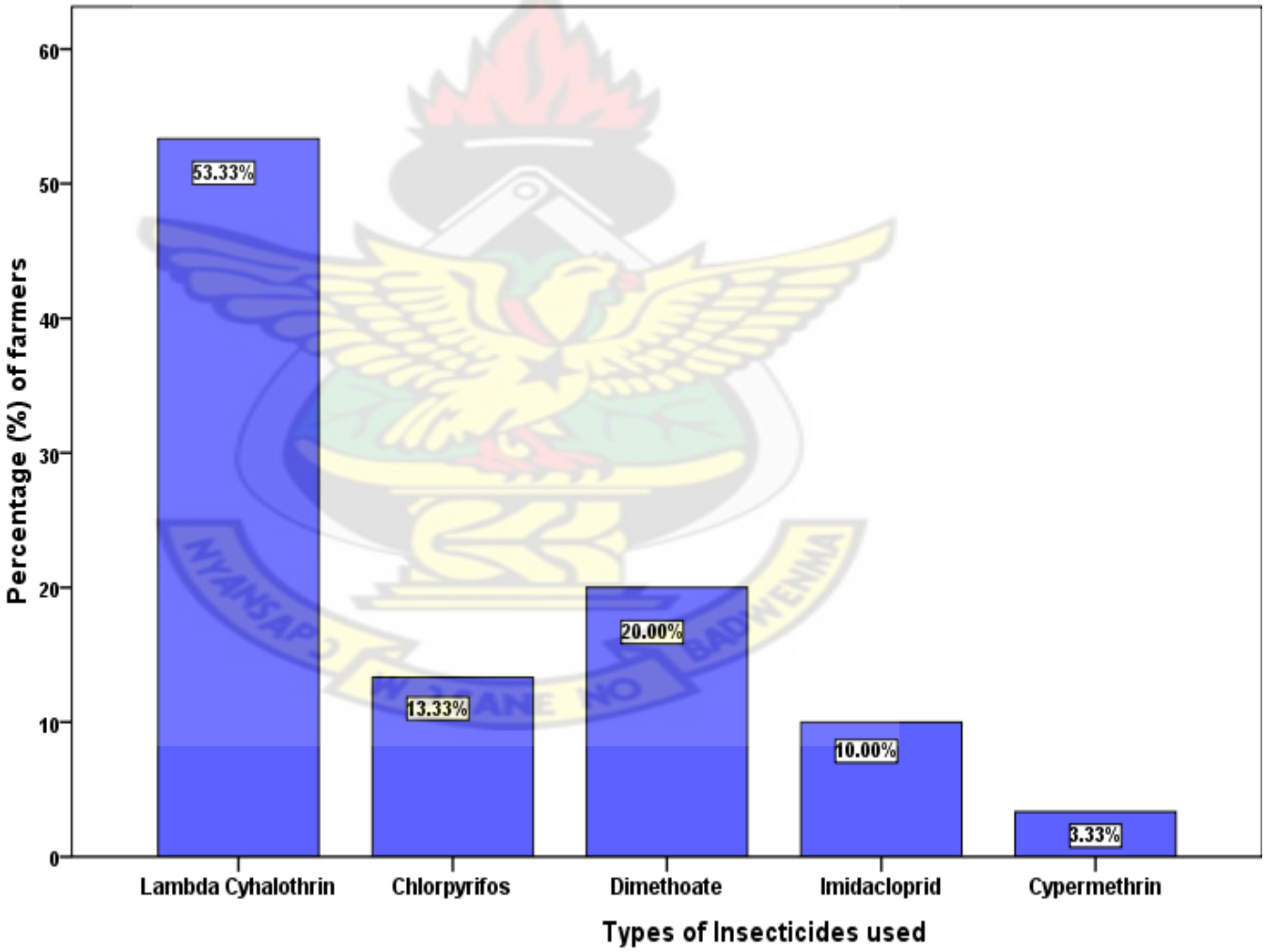


**Fig. 3 Percentage of farmers applying the indicated insecticides on orange trees**

The results of the level of education of farmers who were interviewed in the Ejisu- Juaben Municipality are presented in Tables 2 and 3. It was observed that twelve farmers had Basic Education and out of that, eight preferred lambda cyhalothrin, two preferred dimethoate, one for chlorpyrifos and imidacloprid each. None of them used cypermethrin.

Only four of the farmers had secondary education. Two of them used dimethoate. One each preferred chlorpyrifos and imidacloprid as insecticide to control pest.



**Table 3: Level of Education of Orange Farmers interviewed in the Ejisu- Juaben Municipality**

| Level of education of farmers | Frequency | Percent (%) |
|---|---|---|
| Basic | 12 | 40.0 |
| Secondary | 4 | 13.3 |
| Illiterate | 14 | 46.7 |
| Total | 30 | 100.0 |

Fourteen of the farmers were illiterate; eight of them used Lambda cyhalothrin, three preferred Imidacloprid, two applied Chlorpyrifos and only one sprayed with Cypermethrin (Table 3). None of them used Dimethoate.

**Table 4: Pearson Chi-square test for the Level of Education of farmers and insecticide used**

| | Value | P – Value |
|---|---|---|
| Pearson Chi-Square | 11.214 | 0.190 |



**Hypothesis**

$H_o$: the use of insecticides does not depend on the level of education of farmers

$H_1$: the use of insecticides depends on the level of education of farmers

The results of time of insecticide application on orange trees are presented in Table 5. Twenty nine farmers sprayed their orange trees in the morning. Only one farmer sprayed the farm in the afternoon. Therefore, majority (96.7%) of the farmers prefer spraying their farm in the morning between 7am and 9am.

**Table 5: Time of insecticide application on Orange trees**

| Time of day | Number of Farmers | Percent (%) |
|---|---|---|
| Morning 7am-9am | 29 | 96.7 |
| Afternoon 12pm | 1 | 3.3 |
| | 30 | 100.0 |

### 4.1.2. The Use of Protective Gears by farmers

Protective gears used by farmers interviewed are presented in Tables 3-8 (Appendix C). Fifty percent (50%) of the respondents used the recommended complete clothing while handling the insecticide. Fifty percent (50%) of the respondents did not use the recommended spraying clothing during spraying and majority of them (80%) did not use gloves during spraying. Forty six percent (46%) of the respondents did not use cloth, nose mask, handkerchief on face during mixing and application of insecticide showing a low awareness of health risks. All farmers contacted (100%) wore some footwear to cover their feet during insecticide spraying even though only 26.7% applicators used the prescribed Wellington boots.

Table 6 shows the type of spraying machines used for spraying the orange trees. Twenty four farmers used Knapsack sprayer (80%), while five farmers used Pressure sprayer (16.7%). Only one farmer used Motorized sprayer (3.3%) (Table 6).

**Table 6: Types of Spraying Machine used by farmers**

| Spraying machine | Frequency | Percent (%) |
|---|---|---|
| Knapsack sprayer | 24 | 80.0 |
| Pressure sprayer | 5 | 16.7 |
| Motorized sprayer | 1 | 3.3 |
| Total | 30 | 100.0 |

The results of the disposal method of used insecticide containers by farmers are presented in Table 8. Twenty three farmers (76.7%) have been throwing the used containers away while five of them (16.7%) bury the used insecticide containers. Only two (6.7%) of the farmers burn the used container.

**Table 7: Methods of disposal of used insecticide containers by farmers**

| Disposal of insecticide containers | Frequency | Percent (%) |
|---|---|---|
| Throw them away | 23 | 76.7 |
| Bury them | 5 | 16.7 |
| Burn them | 2 | 6.7 |
| Total | 30 | 100.0 |

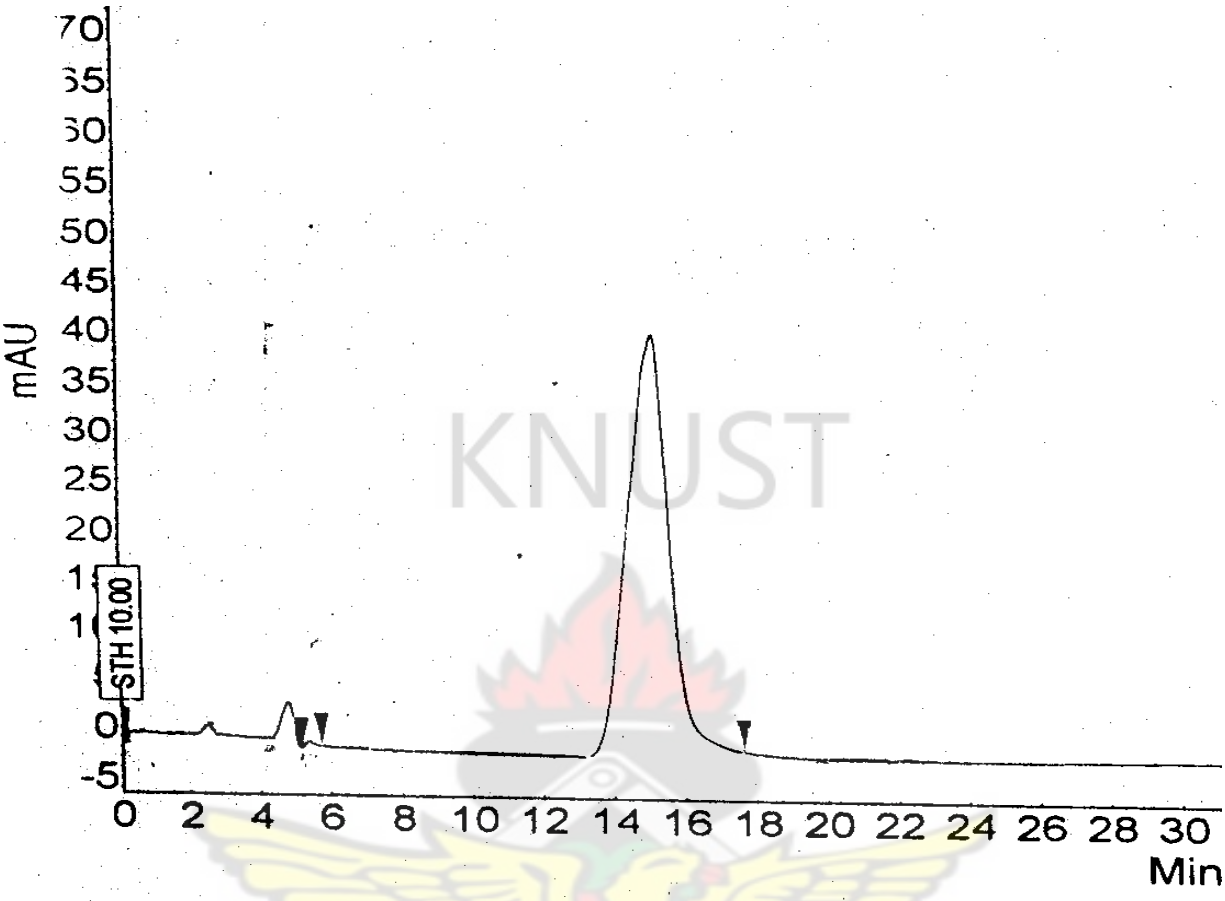


**Fig. 4 Chromatogram of pure Lambda cyhalothrin**
**Retention time of pure Lambda cyhalothrin: 15.20mins.**

Figure 4 shows the chromatogram of pure Lambda cyhalothrin insecticide after it had been run on the mobile phase in the Varian Inc. Liquid Chromatograph. The pure Lambda cyhalothrin peak was obtained at a retention time of 15.20 minutes.

Figure 5 shows the calibration curve obtained for the various concentrations prepared from the pure Lambda cyhalothrin insecticide. The equation of the regression line was given as

$Y = 3085 X + 0.0294$

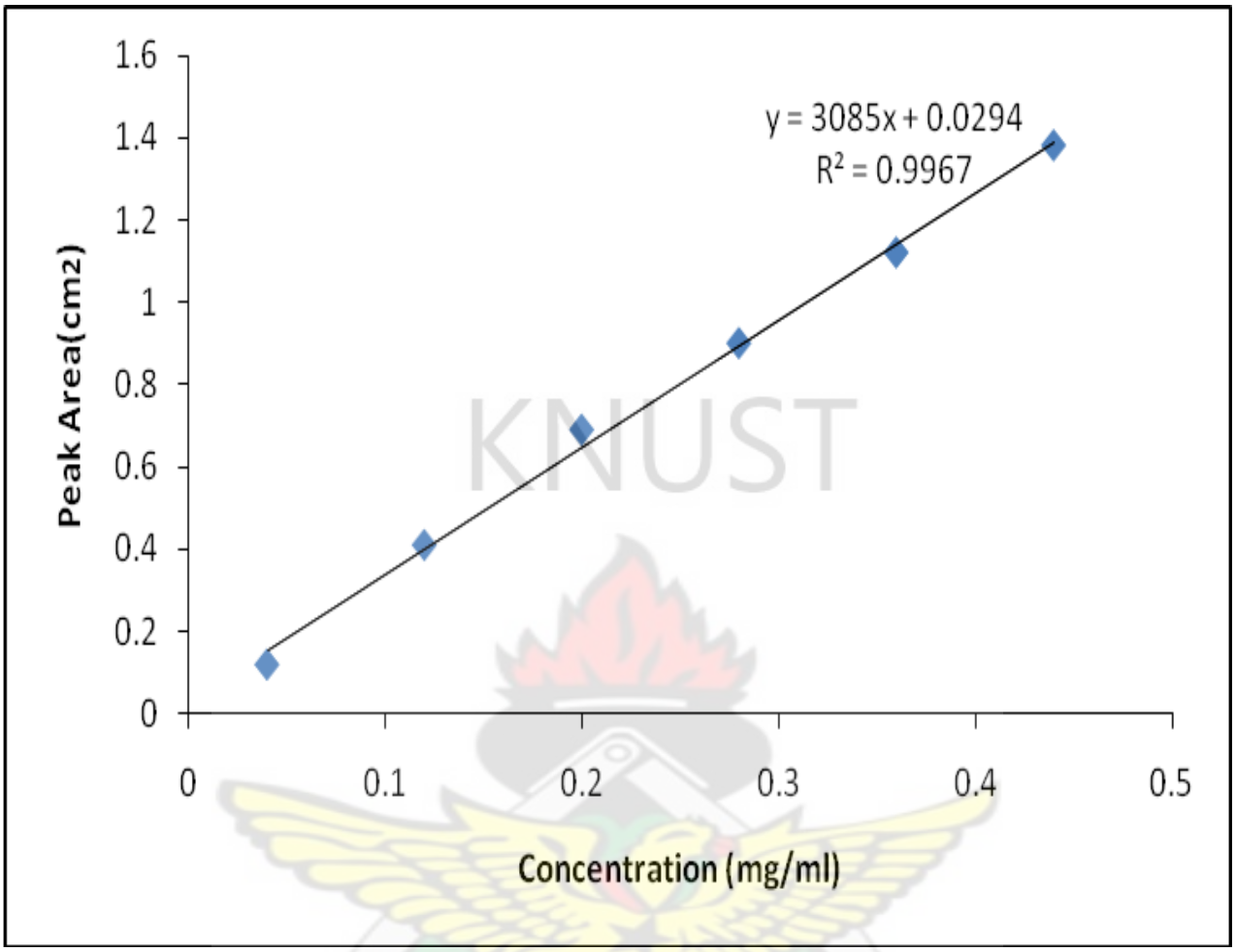


**Fig. 5. Calibration curve for Lambda cyhalothrin standards**

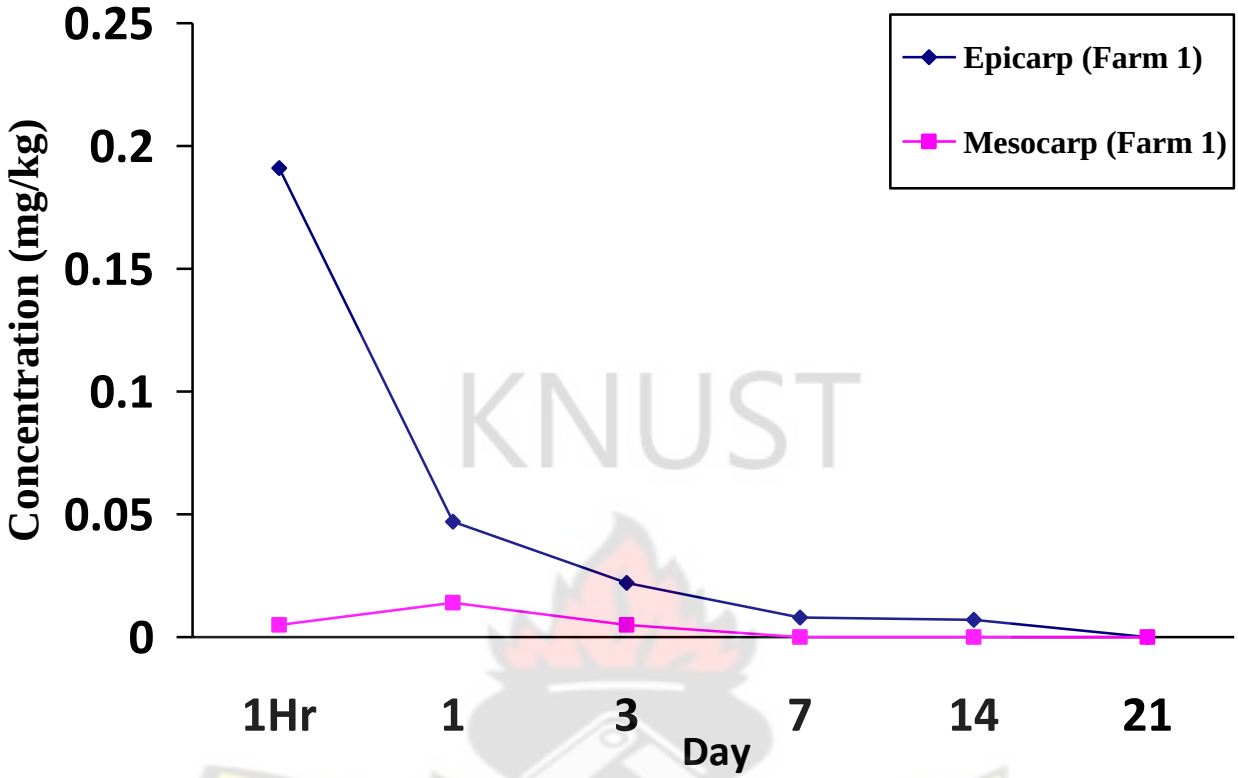


**Fig. 6. Record of amount of insecticide residue detected in oranges from Farm 1 up to 21 days application**

The results of the amount of insecticide residues detected in oranges from Farm 1 are given in Figure 6.

The residue level in the epicarp was high (0.191 mg/kg) one hour after insecticide application but declined sharply on the following day to 0.047 mg/kg and no residue was detected after 21 days.

The residue level in the mesocarp one hour after application was low (0.005 mg/kg). There was a slight rise on the following day (0.014 mg/kg) but declined on the third day to 0.005 mg/kg and virtually no residue was detected on the seventh day and thereafter.

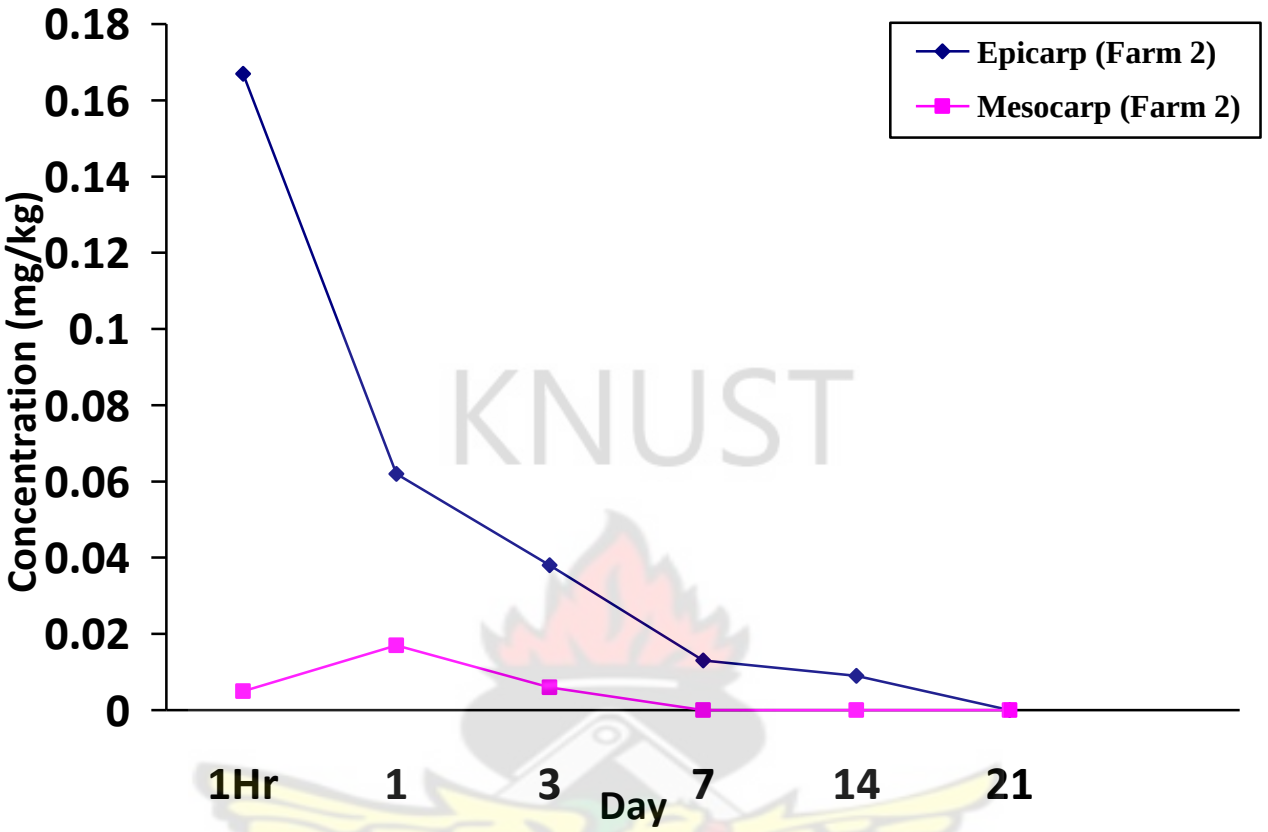


**Fig. 7 Record of insecticide residue detected in oranges from Farm 2 up to 21 days after application.**

The amount of insecticide residue recorded in oranges from Farm 2 is shown in Figure 7. The residue level in the epicarp was 0.167 mg/kg one hour after insecticide application. The level declined sharply to 0.062 mg/kg on the following day which continued to decrease steadily to day three (0.038 mg/kg), to day seven (0.013 mg/kg) and finally no residue was detected 21 days afterwards.

The insecticide residue level in the mesocarp, one hour after insecticide application was (0.005 mg/kg). The residue level increased to 0.017 mg/kg on the following day but dropped on the

third day to 0.006 mg/kg. Residue levels were below detection limit after the seventh day and afterwards.

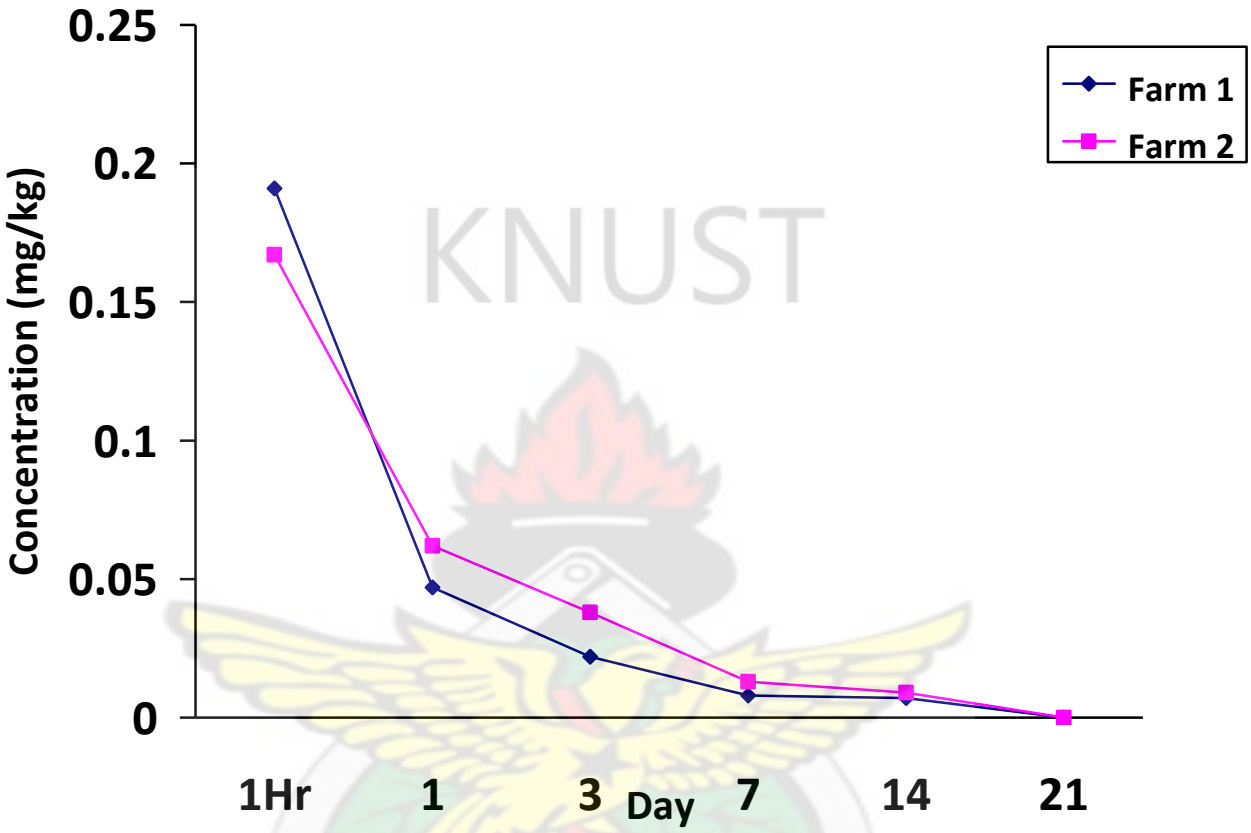


**Fig. 8 Comparative insecticide residue in epicarp of orange from farms 1 and 2 up to 21 days after application**

Results of the epicarp analysis for farms 1 and 2 is indicated in Fig. 8. The insecticide residue level of the insecticide after one hour of application was higher (0.191 mg/kg) in the epicarp of farm 1 than that in farm 2 (0.167 mg/kg).

On day one, the residue level in Farm 1 dropped to 0.047 mg/kg more than in Farm 2 (i.e. 0.062 mg/kg). The residue level further dropped to 0.022 mg/kg on day three, 0.008 mg/kg on day 7, and 0.007 mg/kg on day 14 for Farm 1. For Farm 2, the residue level was 0.038 mg/kg on day

three, 0.013 mg/kg on day 7, and 0.009 mg/kg on day 14. For both farms, no residue was detected on day 21.

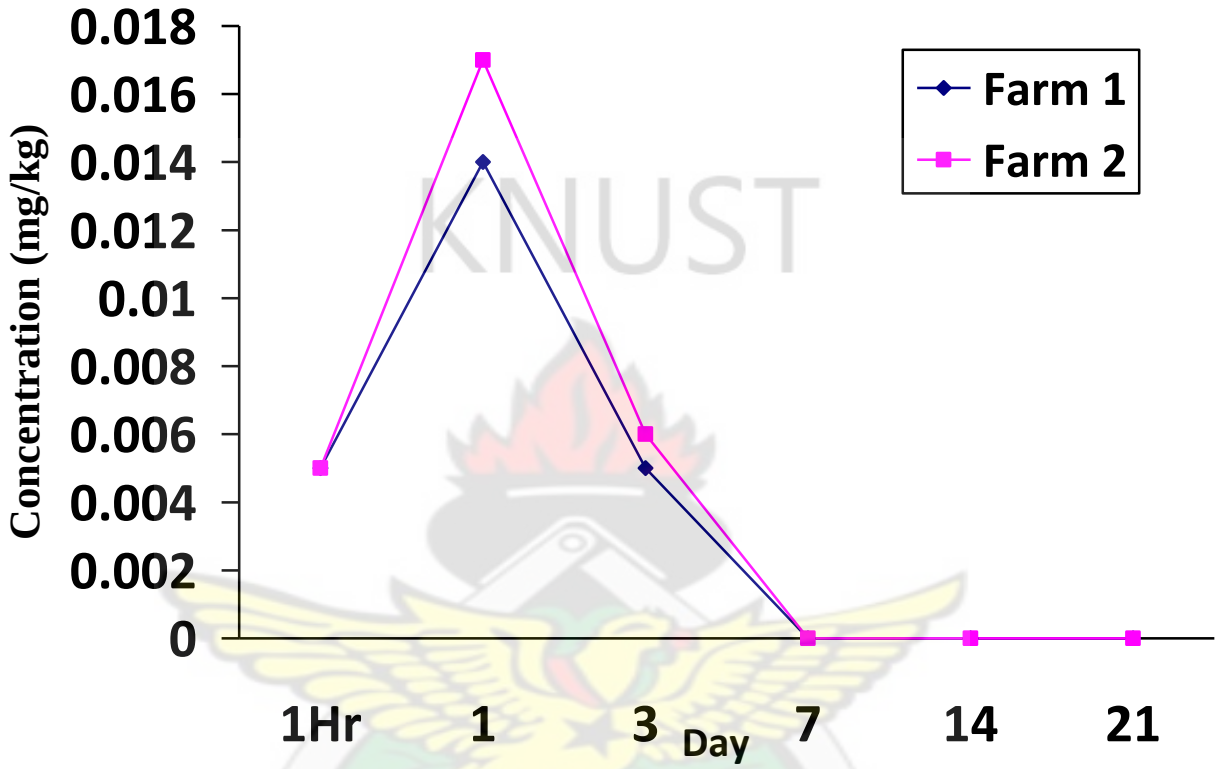


**Fig. 9.Comparative insecticide residue in mesocarp of oranges from farms 1 and 2 up to 21 days**

From Figure 9, the residue level after 1hour of application was 0.005 mg/kg for both farms. However, on day one, the residue level rose in both farms with farm 2 slightly higher (0.017 mg/kg) than in farm 1 (0.014 mg/kg). The residue level then dropped to 0.005 mg/kg and 0.006 mg/kg for farms 1 and 2 respectively on the third day. No residue was detected after seven days.

## 4.2 SAMPLE CALCULATIONS

### 4.2.1 Amount of Insecticide Residues Determined in Oranges Analysis

**Farm 1: Epicarps (Ep)**

Weight of **Ep (Day 0 after insecticide application)** = 383.010 g = 0.383010 kg

Peak area = 0.25 $cm^2$

From calibration graph

Y = 3085x + 0.029

0.25 = 3085x + 0.029

X = 0.0000732 g/ml

Where x is the concentration of lambda cyhalothrin in extract Ep (day 0)

The mass of insecticide = 0.0000732 g = 0.0732 mg i.e. 0.0000732 g/ml = 0.0732 mg/ml

But weight of Epicarp (day 0) = 0.383010 kg

Hence the amount of insecticide residue in Epicarp (day 0)

= (0.0732 mg / 0.383010 kg)

= 0.1911 mg/kg

**Farm 1: Mesocarps (Ms)**

Weight of **Ms (Day 0 after insecticide application)** = 623.926 g = 0.623926 kg

Peak area = 0.12 $cm^2$

From calibration graph

Y = 3085x + 0.029

0.12 = 3085x + 0.029

X = 0.0000294 g/ml

Where x is the concentration of lambda cyhalothrin in extract Ms (day 0)

The mass of insecticide = 0.0000294 g = 0.0294 mg i.e. 0.0000294 g/ml = 0.0294 mg/ml

But weight of Mesocarp (day 0) = 0.623926 kg

Hence the amount of insecticide residue in Mesocarp (day 0)

= (0.0294 mg / 0.623926 kg)

= 0.0471 mg/kg

## 4.3. VALIDATION OF ANALYTICAL METHOD

### 4.3.1. Repeatability (Within- run Precision) of HPLC Method

**TABLE 8: Repeatability of HPLC method for concentration 0.20 mg/ml**

| **Number** | **Peak area ($cm^2$)** | **$x - \bar{x}$** | **$(x - \bar{x})^2$** |
|---|---|---|---|
| 1 | 0.69 | 0.00 | 0.0000 |
| 2 | 0.70 | 0.01 | 0.0001 |
| 3 | 0.69 | 0.00 | 0.0000 |
| 4 | 0.68 | -0.01 | 0.0001 |
| 5 | 0.69 | 0.00 | 0.0000 |
| Total | 3.45 | | 0.0002 |

$\bar{x}$ Represents the mean

$\bar{x}$ = (3.45) / 5 = 0.69

SD = $\sqrt{}$ {$\sum$ $(x - \bar{x})^2$ / n-1}

= $\sqrt{}$ (0.0002 / 4)

= 0.0071

RSD or CV = (SD × 100) / $\bar{x}$

= (0.0071 × 100) / 0.69

= 1.03%

**Table 9: Repeatability of HPLC method for concentration 0.36 mg/ml**

| **Number** | **Peak area ($cm^2$)** | **x - $\bar{x}$** | **(x -$\bar{x}$ )$^2$** |
|---|---|---|---|
| 1 | 1.11 | -0.01 | 0.0001 |
| 2 | 1.12 | 0.00 | 0.0000 |
| 3 | 1.11 | -0.01 | 0.0001 |
| 4 | 1.12 | 0.00 | 0.0000 |
| 5 | 1.13 | 0.01 | 0.0001 |
| Total | 5.6 | | 0.0003 |

$\bar{x}$ Represents the mean

$\bar{x}$= (5.6) / 5 = 1.12

SD = √ {∑ (x -$\bar{x}$ )$^2$ / n-1}

SD = √ (0.0003) / 4

SD = 0.0086

RSD = (SD x 100%) / $\bar{x}$

= (0.0086 ×100) / 1.12

= 0.76%

**Table 10: Repeatability of HPLC method for concentration 0.44 mg/ml**

| Number | Peak area ($cm^2$) | x- $\bar{x}$ | (x -$\bar{x}$ )$^2$ |
|---|---|---|---|
| 1 | 1.37 | -0.01 | 0.0001 |
| 2 | 1.38 | 0.00 | 0.0000 |
| 3 | 1.37 | -0.01 | 0.0001 |
| 4 | 1.39 | 0.01 | 0.0001 |
| 5 | 1.39 | 0.01 | 0.0001 |
| Total | 6.9 | | 0.0004 |



$\bar{x}$= (6.9) / 5 = 1.38

SD = $\sqrt{}$ {$\sum$ (x -$\bar{x}$ )$^2$ / n-1}

= $\sqrt{}$ 0.0004/ 4

SD = 0.01

RSD = (SD x 100%) / $\bar{x}$

= (0.01 x 100) / 1.38

= 0.72%

**Table 11: Summary of Repeatability of Method Results**

| Concentration (mg/ml) | Mean | Standard Deviation | RSD % |
|---|---|---|---|
| 0.20 | 0.69 | 0.0071 | 1.03 |
| 0.36 | 1.12 | 0.0086 | 0.76 |
| 0.44 | 1.38 | 0.010 | 0.72 |



### 4. 3.2. Reproducibility of Method (Between- run Precision)

**TABLE 12: Reproducibility of HPLC Method**

| | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| **Determination** | **Peak area $cm^2$** | **Peak area $cm^2$** | **Peak area $cm^2$** |
| 1 | 0.40 | 0.41 | 0.40 |
| 2 | 0.41 | 0.42 | 0.40 |
| 3 | 0.40 | 0.41 | 0.42 |
| 4 | 0.41 | 0.40 | 0.41 |
| 5 | 0.41 | 0.40 | 0.41 |

**Table 13: Summary of Reproducibility of Method Results**

| **Concentration** | **0.00012g/ml** | | |
|---|---|---|---|
| **Day(s)** | Day 1 | Day 2 | Day 3 |
| **Mean ($\bar{x}$)** | 0.41 | 0.41 | 0.41 |
| **Standard Deviation (SD)** | 0.0070 | 0.0086 | 0.0086 |
| **RSD (%)** | 1.7 | 2.0 | 2.0 |



## 4.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ) Calculations

LOD and LOQ are based on the slope and standard deviation (SD) of the response

$LOD = (3.3\ \delta) / S$

Where, S = slope of the calibration graph.

$\delta$ = SD of the response= the residual standard deviation ($S_{res}$) of a regression line.

$S_{res} = \sqrt{\{\sum (y - y_{est})^2 / n - 2\}}$

Y= observed Y values on vertical axis

$Y_{est}$ = Y value estimated

$Y_{est}$ = Values of Y calculated from the equation of regression line, i.e.

Equation y= 3085x + 0.029

For x = 0.00004(various concentrations for calibration graph)

$Y_{est} = 3085(0.00004) + 0.029 = 0.1524$

For x = 0.00012

$Y_{est} = 3085(0.00012) + 0.029 = 0.3992$

For x = 0.00020

$Y_{est} = 3085(0.00020) + 0.029 = 0.646$

For x = 0.00028

$Y_{est} = 3085(002996) + 0.029 = 0.8928$

For x = 0.00036

$Y_{est} = 3085(0.00036) + 0.029 = 1.1396$

For x = 0.00044

$Y_{est} = 3085(0.00044) + 0.029 = 1.3864$

**TABLE 14: LOD and LOQ CALCULATIONS**

| **Y** | **$Y_{est}$** | **$Y - Y_{est}$** | **$(Y - Y_{est})^2$** |
|---|---|---|---|
| 0.12 | 0.1524 | - 0.0324 | 0.00104976 |
| 0.41 | 0.3992 | 0.0108 | 0.00011664 |
| 0.69 | 0.646 | 0.044 | 0.001936 |
| 0.90 | 0.8928 | 0.0072 | 0.00005184 |
| 1.12 | 1.1396 | -0.0196 | 0.00038416 |
| 1.38 | 1.3864 | - 0.0064 | 0.00004096 |

$\sum (Y - Y_{est})^2 = 0.00357936$

Now,

Residual standard deviation, $S_{res} = \sqrt{\{\sum (Y - Y_{est})^2\}} / n - 2$

$= \sqrt{\{(0.00357936) / (6 - 2)\}}$

$= \sqrt{\{0.00357936\}} / 4$

$= \sqrt{0.00089484}$

$\Longrightarrow \quad S_{res} = \delta = 0.029913876$

$LOD = (3.3\ \delta) / S = (3.3 \times 0.029913876) / 3085$

Where, S = Slope

$= 0.000031998$ g/ml

LOD = 0.031998 mg/ml

$LOQ = (10\ \delta) / S = (10 \times 0.029913876) / 3085$

$= 0.000096965$ g/ml

LOQ = 0.096965 mg/ml.

# CHAPTER FIVE

## DISCUSSION

### 5.1: Field Survey

#### 5.1.1 Types of Insecticide used by farmers

Farmers used Lambda cyhalothrin because they have observed that the insecticide has the ability of killing many insects which are pests of citrus. According to IPCS (1984), Lambda cyhalothrin has a high level of activity against a wide range of Lepidoptera, Hemiptera, Diptera and Coleoptera spp. It is extremely effective against a number of insects resistant to standard treatments such as organochlorines and organophosphates (IPCS, 1984). Durkin (2010), also explained that Lambda cyhalothrin is an effective insecticide and is highly toxic to insects as well as other terrestrial arthropods. Again, farmers confirmed that Lambda cyhalothrin is mostly available on the market and highly recommended by most Agrochemical dealers.

The use of Dimethoate was as a result of it being able to drive insects away. Cypermethrin was not preferred by the farmers because it was costly and not recommended by the dealers of Agrochemicals. However, the few farmers (3.33%) who used cypermethrin confirmed that apart from being costly, it was effective in killing insects of citrus. Cypermethrin kills insects by disrupting the normal functioning of the nervous system according to Cantalamessa, (1993).

#### 5.1.2: Level of Education and Pesticide application on farms

All farmers interviewed used insecticides to spray their orange trees whether educated or not. Table 4, presents a chi-square test of independence for the stated hypothesis on the Level of

education of farmers and the use of insecticides among farmers in Ejisu - Juaben Municipality. The Chi - square statistic with p-value of 0.190 is higher than the level of significance which is 0.05. This suggests that the test is not significant; therefore we fail to reject the null hypothesis. This brings out the implication that the use of insecticides does not depend on the level of education of the farmers in the Municipality.



### 5.1.3: Time of insecticide application on Orange trees

Most farmers (96.7%) interviewed sprayed their orange trees in the morning between 7am and 9am because they have observed that most insects are active in the morning. Only one farmer sprayed the trees in the afternoon due to the closeness of the farm to the house. This reason agrees with that of Banerjee *et al.* (2003), who reported that pesticides are effective in the morning or late afternoon when insects are most active. Applying on hot, sunny days leads to quick evaporation resulting in reduced pest control.

### 5.1.4: The Use of Protective gear

More than half (64.3%) of the farmers contacted were not using the recommended protective gears during insecticide application. They were not using recommended protective gears either due to discomfort or due to ignorance. Similar findings were reported from the study conducted by Dogra *et al.* (1998). Putnam *et al.* (1983), reported that the use of rubber gloves reduce pesticide exposure, but concluded that gloves did not completely eliminate exposure when working with vegetable crops treated with nitrofen. Farmers could not afford to buy protective

gear making it more risky when it comes to pesticide application. Dinham (1996), reported that health problems associated with the use of pesticides in developing countries are magnified by farmers' inability to afford protective clothing, high rates of illiteracy and virtual impossibility of wearing protective clothing in hot climates.

### 5.1.5: Spraying Machine used by farmers

Eighty percent of the respondents were using Knapsack Sprayer due to its easy availability and suitability for their crops in spraying. A study conducted by McAuliffe and Gray (2002), showed that interest has remained high with this type of equipment as its versatility in use with different types of pesticides suits the requirements and resources of small-scale farmers aiming to increase agricultural productivity under harsh conditions in developing nations. New innovations in plastics and metals, making sprayers lighter in weight and more efficient in pumping, have made knapsack sprayers easier to use, particularly in areas with excessive heat and difficult terrain (McAuliffe and Gray, 2002).

However, only 3.3% farmers were using motorized sprayers due to the difficulties they find in using these sprayers. The motorized sprayer is heavy to carry at the back and the sound it makes when in operation makes it uncomfortable to use.

### 5.1.6: Disposal of used insecticide containers

Proper rinsing is necessary so that insecticide may not contaminate the surrounding environment and ground water. Most of the insecticide applicators (76.7%) threw the empty containers away without proper rinsing after insecticide application due to illiteracy and ignorance. Some farmers (16.7%) buried used containers with the idea that the container will decompose in the soil and

the left over chemical in it will drain into the soil without any negative impact. Few applicators (6.7%) burnt used containers believed burning will destroy the containers with no possible negative effect on the environment.

## 5.2: HPLC Analysis

### 5.2.1: Chromatographic conditions

The HPLC conditions that were finally used comprised mobile phase 60% acetonitrile, 20% methanol and 20% water mixture at wavelength 254 nm, reversed phase $C_{18}$ column, flow rate of 1.0ml/minute, sensitivity 0.500, Attenuation 0. Temperature was maintained at ambient temperature and mean retention time obtained was 15.20 $\pm$ 0.11 minutes.

### 5. 2.2: Insecticide residues in extracts from Epicarp and Mesocarp from Farm 1

The large amount of insecticide that was detected in the epicarps (1 hour) after application, could be attributed to the thickness of the epicarps. This agrees with the findings of Rajeev (2011) who stated that blood oranges have thick epicarp and have the ability to retain more insecticides. Also the epicarps were in direct contact with the insecticides during the application and therefore absorbed more of the insecticide. On the third, seventh and fourteenth days, the residue levels reduced drastically. No residue was detected after 21 days. The decline could be as a result of the insecticide being broken down into different by-products. Mueller-Beilschmidt (1990), reported that pyrethroids breakdown quickly in direct sunlight, air and water usually just a few days after application.

The low residue level detected in the mesocarp one hour after application could be due to the thickness of the epicarp but the slight rise on the following day indicates that the insecticide penetrated into the mesocarp of the oranges. There was virtually no residue detected on the seventh and the subsequent days indicating that the insecticide might have degraded before the seventh day. Similar findings were reported from the study conducted by Banerjee *et al.* (2006) on grapes (*Vitis vinifera*).



### 5.2.3: Insecticide residues in extracts from Epicarp and Mesocarp from Farm 2

Lambda cyhalothrin insecticide residues detected in the epicarps and the mesocarps from Farm 2 as compared to Farm 1 were low. The results obtained showed that all the orange fruits analyzed were safe for consumption since the residues detected were below the FAO/WHO (2010) maximum residue limit of 0.2 mg/kg established (2010). A study conducted on wheat by Kimkaid and Sapiets (1986), with lambda cyhalothrin application also saw no measurable residues in the mature grain after 28 days. A similar work was done by Fitzpatrick (1984), on cotton with Lambda cyhalothrin application. After 20 days, a lambda cyhalothrin residue 0.01 mg/kg was detected in only one cotton seed sample; all other samples had no residues. These indicate that crops sprayed with Lambda cyhalothrin can be consumed after twenty one (21) days of application.

### 5.2.4: Insecticide Residues in extracts of Epicarp from Farms 1 and 2

From figure 4.6, even though the residue levels were high for both farms, they were below FAO/WHO (2010) Maximum Residue Limit of 0.2 mg/kg. On day one, the residue level in the epicarp of orange fruits from Farm 1 dropped more than in Farm 2. The orange fruits from farm

2 were of the Blood variety having thicker epicarp than those from farm 1 which belong to the Valencia variety. More of the insecticide might have been taken up by the epicarp from farm 2 therefore retaining more residues than those from farm 1. The residue level further dropped on day three, seven and on day fourteen for both farms with farm 2 slightly higher than farm 1. For both farms, no residue was detected on day 21. These low levels of residual insecticide in the fruits analysed could be attributed to many factors. There is a probability of the insecticide being washed away by rainfall during the time the fruits were on the farm after they have been sprayed with lambda cyhalothrin. There was also the probability of insecticide being broken down as a result of direct interaction with sunlight, moisture or air.



**5.2.5: Insecticide residues from extracts of Mesocarp from Farms 1 and 2**

The residue levels after 1hour of application was 0.005 mg/kg for both farms (Fig. 9). However, on day one, the residue level rose in both farms with farm 2 slightly higher (0.017 mg/kg) than in farm 1 (0.014 mg/kg). The residue level then dropped to 0.005 mg/kg and 0.006 mg/kg for farms 1 and 2 respectively on the third day. No residue was detected on the seventh day and afterwards. Mesocarp of oranges is therefore safe for consumption after the seventh day of lambda cyhalothrin insecticide application. Residues of lambda cyhalothrin according to Banerjee *et al.* (2006), were lost with pre-harvest intervals (PHI) of 12.0-12.5 days and 15.0-15.5 days respectively. The work of Soliman (2011), on cowpea at different days of pre-harvest interval also saw that lambda cyhalothrin residues within 7 days were all above the maximum residue limit of 0.2 mg/kg. However, after 10 days the residues were below the maximum residue limit.

# CHAPTER SIX

## CONCLUSION AND RECOMMENDATION

### CONCLUSION

The insecticides used to spray oranges in the Ejisu – Juaben Municipality were found to be Lambda-cyhalothrin, Dimethoate, Chlorpyrifos, Imidacloprid and Cypermetrhin. About 53.33% of farmers interviewed used Lambda cyhalothrin insecticide and Cypermethrin was the least frequently used insecticide representing 3.33% of the farmers.

Lambda cyhalothrin degraded considerably in the oranges. Therefore oranges treated with lambda cyhalothrin may be consumed safely after 10-14 days for mesocarp and 15 days or more for epicarp.

Out of 24 composite samples analysed, 66.67% of the samples were contaminated with lambda cyhalothrin residues. However, the residues level detected in them were below FAO/WHO maximum residue limits of 0.2 mg/kg for citrus. These samples may not pose health hazards to the consumers.

Eating of fruits sprayed with pesticides without taking into account their withholding periods may lead to accumulation of residues higher than the tolerance limits. This is mainly attributed to illiteracy of the farmers and lack of effective legislation in the country.

## RECOMMENDATIONS

There should be increased public awareness on the right methods of pesticide application, insecticide residues and their effects on humans.

In view of an increasing trend in insecticide use in Ghana, continuous monitoring for insecticides and other pesticides residues is needed in crops especially fruits in order to protect the end user from health hazards involved in the misuse of insecticides and to generate base line data upon which future plans could be developed.

Further studies should be carried out in other orange growing areas in the Municipalities and Districts to determine insecticide residue levels in the orange fruits.

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## APPENDICES

### APPENDIX A: Questions

**INSECTICIDE USE SURVEY IN EJISU-JUABEN MUNICIPALITY, GHANA, 2011**

Please note that the information given will be treated as confidential and will not be used for any other purpose except for the intended purpose.



**SECTION 1: FARM IDENTIFICATION**

| | | |
|---|---|---|
| 01 | TOWN | |
| 02 | LEVEL OF EDUCATION | Basic [ ]<br>Secondary [ ]<br>University [ ]<br>Illiterate [ ] |

**SECTION 2: FARM OPERATIONS**

| NO | QUESTIONS | RESPONSE OPTIONS |
|---|---|---|
| 03 | Which insecticide do you use for insects or pests on your fruits? | 1. Cymethoate [ ]<br>2. Lambda super [ ]<br>3. Consider [ ]<br>4. Cypadem [ ]<br>5. Desban [ ] |

| | | |
|---|---|---|
| | | 6. Other ……..<br>(specify) |
| 04 | What type of spraying machine do you use? | 1. Knapsack sprayer [ ]<br>2. Pressure sprayer [ ]<br>3. Motorized sprayer **[ ]**<br>4. Other …….<br>(specify) |
| 05 | Who does the spraying? | 1. Self [ ]<br>2. Employ services of others [ ] |
| 06 | At what time of the day do you spray? | 1. Morning [ ]<br>2. Afternoon [ ]<br>3. Evening [ ]<br>4. Other ……..<br>(specify) |

**SECTION 3: PRECAUTIONS IN THE USE OF INSECTICIDES**

| NO | QUESTIONS | RESPONSE OPTIONS |
|---|---|---|
| 07 | What clothes do you wear when spraying your crops? | 1. Short sleeves and trousers [ ]<br>2. Long sleeves and trousers [ ]<br>3. Overall [ ]<br>4. Jacket [ ]<br>5. Other ……<br>(specify) |

| | | |
|---|---|---|
| 08 | What do you use to protect your hands when spraying? | 1. Handkerchief [ ]<br>2. Cloth [ ]<br>3. Gloves [ ]<br>4. Polythene bag [ ]<br>5. Other ……<br>(specify) |
| 09 | What do you wear to protect your feet? | 1. Canvas [ ]<br>2. Shoe [ ]<br>3. Wellington boot [ ]<br>4. Other ……..<br>(specify) |
| 10 | What do you use to cover your nose and mouth when spraying? | 1. Nose mask [ ]<br>2. Handkerchief [ ]<br>3. Cloth [ ]<br>4. Face shield [ ]<br>5. Other ……<br>(specify) |
| 11 | How do you discard the containers after using the insecticide? | 1. Throw them away [ ]<br>2. Bury them [ ]<br>3. Burn them [ ]<br>4. Other …….<br>(specify) |

**APPENDIX B: Plates of insecticides used by farmers in the Ejisu-Juaben Municipality**

**Plate 2: Dimethoate** **Plate 3: Cypermethrin**

**Plate 4 Chlorpyrifos** **Plate 5 Imidacloprid**

**APPENDIX C: Analysis of interview conducted**

**Table 1: Percentages of insecticides used by farmers in the Ejisu - Juaben Municipality**

| Insecticide used | Frequency | Percent (%) |
|---|---|---|
| Lambda Cyhalothrin | 16 | 53.3 |
| Chlorpyrifos | 4 | 13.3 |
| Dimethoate | 6 | 20.0 |
| Imidacloprid | 3 | 10.0 |
| Cypermethrin | 1 | 3.3 |
| Total | 30 | 100.0 |



**Table 2: Towns visited and the Level of Education of Farmers**

| Towns visited | Level of education of farmers | | | Total |
|---|---|---|---|---|
| | Basic | Secondary | Illiterate | |
| Abankro | 2 | 0 | 1 | 3 |
| Boamadumasi | 2 | 1 | 4 | 7 |
| Boankra | 2 | 0 | 1 | 3 |
| Duampompo | 1 | 1 | 2 | 4 |
| Ejisu | 1 | 1 | 3 | 5 |
| Juaben | 2 | 1 | 1 | 4 |
| Kubease | 1 | 0 | 1 | 2 |
| Kwamo | 1 | 0 | 1 | 2 |
| Total | 12 | 4 | 14 | 30 |

**Table 3: Protective clothes worn during insecticides application**

| Protective clothes worn | Frequency | Percent (%) |
|---|---|---|
| Long sleeves and trousers | 8 | 26.7 |
| Overall | 7 | 23.3 |
| Any clothes | 15 | 50.0 |
| Total | 30 | 100.0 |



**Table 4: Protective Nose cover**

| | Frequency | Percent (%) |
|---|---|---|
| Cloth | 7 | 23.3 |
| Nose mask | 5 | 16.7 |
| Handkerchief | 2 | 6.7 |
| No cover | 16 | 53.3 |
| Total | 30 | 100.0 |

**Table 5: Level of education and Nose cover**

| | | Nose and mouth cover | | | | Total |
|---|---|---|---|---|---|---|
| | | Cloth | Nose mask | Handkerchief | No cover | |
| Level of education | Basic | 4 | 1 | 0 | 7 | 12 |
| | Secondary | 0 | 4 | 0 | 0 | 4 |
| | Illiterate | 2 | 1 | 2 | 9 | 14 |
| Total | | 6 | 6 | 2 | 16 | 30 |

**Table 6: Level of education and footwear used during spraying**

| | | Footwear used during spraying | | | |
|---|---|---|---|---|---|
| | | Canvas | Wellington boot | Any available footwear | Total |
| Level of education | Basic | 2 | 1 | 9 | 12 |
| | Secondary | 0 | 1 | 3 | 4 |
| | Illiterate | 3 | 6 | 5 | 14 |
| Total | | 5 | 8 | 17 | 30 |



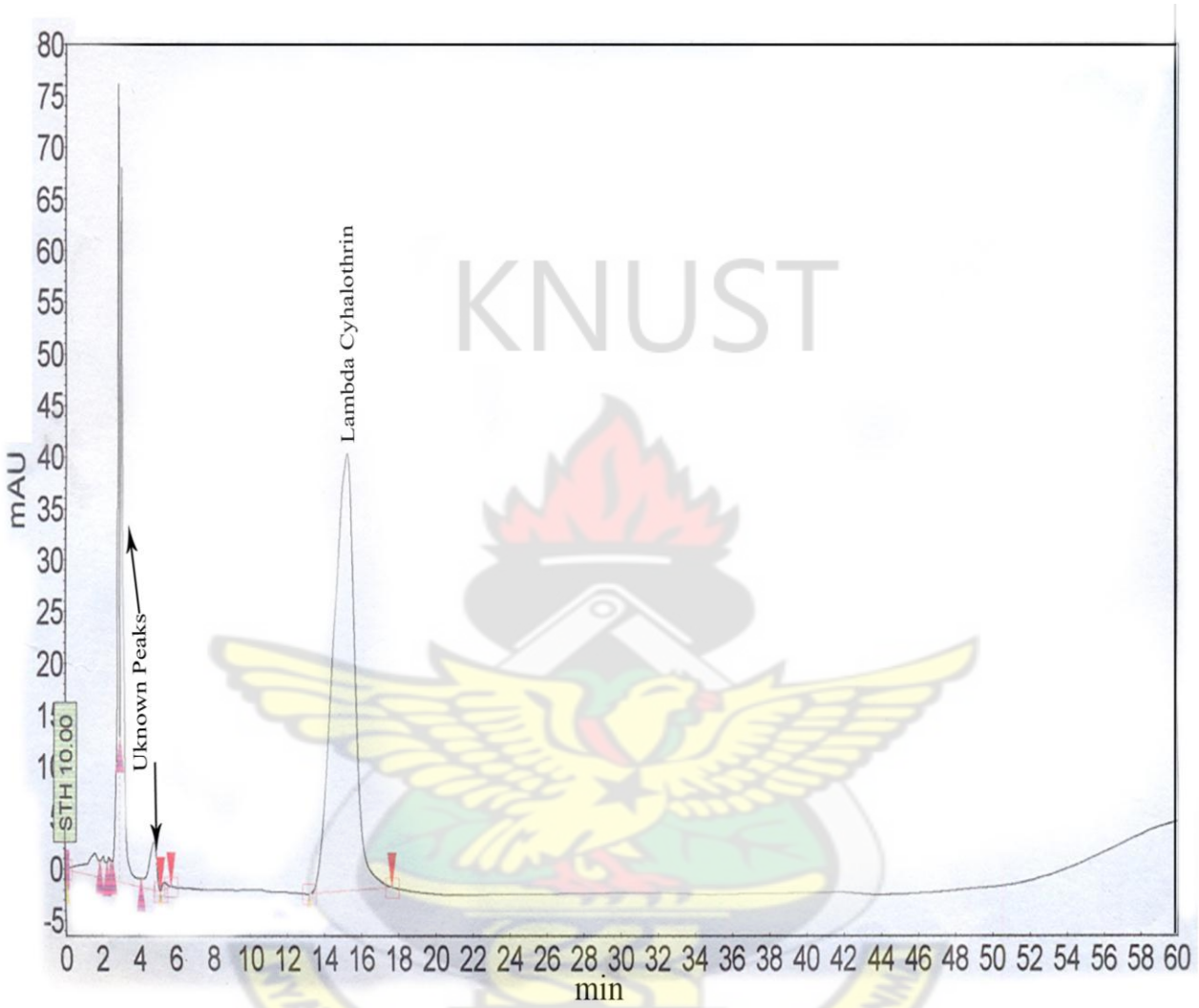
**Table 7: Pearson Chi-square test for the Level of Education and footwear used**

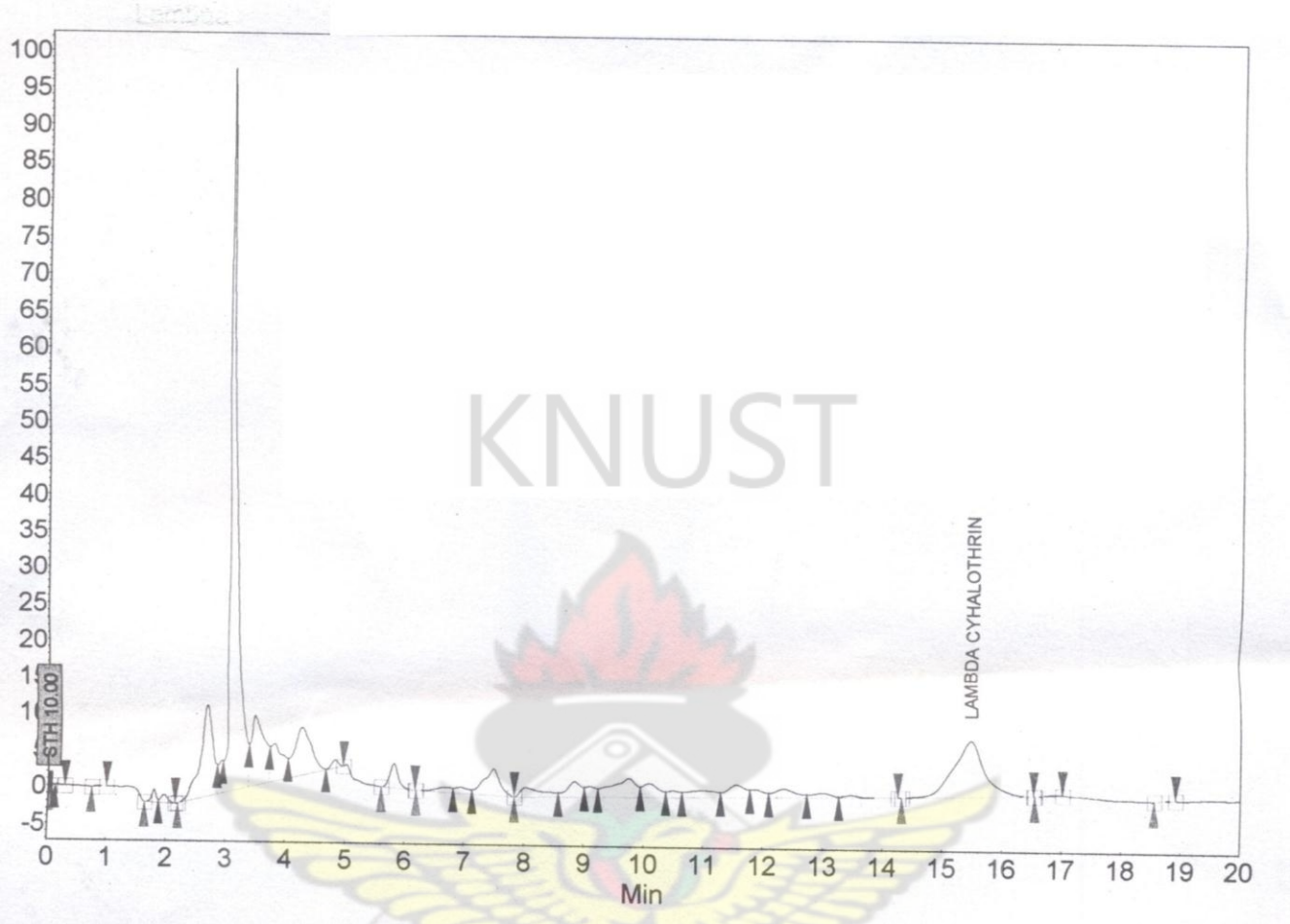
| | Value | P-Value |
|---|---|---|
| Pearson Chi-Square | 5.784 | 0.216 |

**Table 8: Protective hand cover used during spraying**

| | Frequency | Percent (%) |
|---|---|---|
| Polythene bag | 5 | 16.7 |
| Gloves | 6 | 20.0 |
| Cloth | 4 | 13.3 |
| No cover | 15 | 50.0 |
| Total | 30 | 100.0 |

**APPENDIX D : Chromatograms of Lambda cyhalothrin in Epicarp and Mesocarp of Orange fruits**




100
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0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Min

**APPENDIX E. Insecticide residue analysis**

**Farm 1. Amount of insecticide residue detected in the Epicarp**

| **Day** | **Amount of insecticide residue (mg/kg)** |
|---|---|
| 0 (1hour) | 0.191 |
| 1 | 0.047 |
| 3 | 0.022 |
| 7 | 0.008 |
| 14 | 0.007 |
| 21 | Nd |

Where; Nd means no lambda cyhalothrin residue was detected

**Farm 1. Amount of insecticide residue detected in the Mesocarp**

| **Day** | **Amount of insecticide residue (mg/kg)** |
|---|---|
| 0(1hour) | 0.005 |
| 1 | 0.014 |
| 3 | 0.005 |
| 7 | Nd |
| 14 | Nd |
| 21 | Nd |

**Farm 2. Amount of insecticide residue detected in the Epicarp**

| Day | Amount of insecticide residue (mg/kg) |
|---|---|
| 0(1hour) | 0.167 |
| 1 | 0.062 |
| 3 | 0.038 |
| 7 | 0.013 |
| 14 | 0.009 |
| 21 | Nd |

**Farm 2. Amount of insecticide residue detected in the Mesocarp**

| Day | Amount of insecticide residue (mg/kg) |
|---|---|
| 0(1hour) | 0.005 |
| 1 | 0.017 |
| 3 | 0.006 |
| 7 | Nd |
| 14 | Nd |
| 21 | Nd |

**Epicarp analysis for Farms 1 and 2**

| | **Farm 1** | **Farm 2** |
|---|---|---|
| **Day** | **Amount of insecticide residue (mg/kg)** | **Amount of insecticide residue (mg/kg)** |
| 0 (1hour) | 0.191 | 0.167 |
| 1 | 0.047 | 0.062 |
| 3 | 0.022 | 0.038 |
| 7 | 0.008 | 0.013 |
| 14 | 0.007 | 0.009 |
| 21 | Nd | Nd |

**Mesocarp analysis for Farms 1 and 2**

| | **Farm 1** | **Farm 2** |
|---|---|---|
| **Day** | **Amount of insecticide residue (mg/kg)** | **Amount of insecticide residue (mg/kg)** |
| 0(1hour) | 0.005 | 0.005 |
| 1 | 0.014 | 0.017 |
| 3 | 0.005 | 0.006 |
| 7 | Nd | Nd |
| 14 | Nd | Nd |
| 21 | Nd | Nd |

**APPENDIX F: Proposed Guidelines for pesticide use**

**Personal safety when using pesticides**

Due to the variable nature of home garden products it is essential that you always read the label carefully and follow the instructions. Pay particular attention to the safety instructions.

- Avoid getting pesticides on your skin unless the product is designed for that purpose, such as with some personal insect repellents.
- Take care not to swallow pesticides, get them in your eyes, or inhale dust, spray or vapour from them.
- Always wear appropriate personal protective equipment if stated on the label.
- Remove protective clothing and then wash your hands with soap before eating, drinking, smoking or going to the toilet, and at the end of the job.
- Some pesticides are sold in, or need to be diluted in, flammable solvents. Handle these pesticides in a well-ventilated area and make sure there are no sources of ignition nearby when using them.
- Tell someone responsible if you feel ill during or after pesticide use, especially if you have been exposed to a pesticide, including by inhalation, skin contact or ingestion. Seek prompt medical attention or call the Poisons Information Centre on 13 11 26 as soon as possible, providing them with the names of the active ingredients in the pesticide.