DETERMINATION OF THE RESIDUES OF LAMBDA CYHALOTHRIN IN OIL PALM AGROSYSTEM

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Abstract

The objective of this study is to validate the method of detection for lambda cyhalothrin in samples of crude palm and palm kernel oil, soil, leaves and water using gas chromatography-microelectron capture detector (GC-μECD). Based on the establish method, a monitoring study was done in the tropical climate condition of Malaysia to evaluate the dissipation pattern and persistence of lambda cyhalothrin in the oil palm agroecosystem. Our results indicated that the mean recoveries of lambda cyhalothrin were found to be 87-90%, 96-109%, 81-105% and 83-90%, respectively, for crude palm and palm kernel oil, leaves, soil and water sample at three levels of concentrations. The limit of quantification of the method was 0.02 mg/kg, while the limit of detection was 0.002 mg/kg. The monitoring study was conducted at Amcorp Plantation in Sepang, Selangor with three experimental plots of control, recommended dosage and double recommended dosage. The spraying application was conducted in triplicate for each plot involved. Lambda cyhalothrin (ALERT 2.8EC) was sprayed on leaves and at recommended (31.5 g a.i./ha) and double recommended (63 g a.i./ha) manufacturer's dosage. Samples of oil palm fruits, leaves, soils and water were collected on day 0 (6 hours after spraying), 1, 3, 5, 7, 9, 10, 12, 14, 16, 18, 20 and 29 days after treatment. Initial depositions of lambda cyhalothrin on foliar surfaces were 0.47 and 0.83 mg/kg, at recommended and double recommended dosages respectively. Lambda cyhalothrin residues were not detected after 14 and 18 days of the recommended and double recommended dosages, respectively. No residue was detected in the harvested oil palm fruits, water and soil samples. The half-life ($t_{1/2}$) value of lambda cyhalothrin was observed to be 3.96 and 4.88 days for recommended and double recommended dosages respectively on leaves of palm oil.

Abstrak

Objektif atau tujuan kajian ini ialah untuk mengesahkan kaedah analisis bagi racun lambda cyhalothrin di dalam sampel minyak sawit mentah dan isirong sawit, tanah, daun dan air menggunakan gas kromatografi-detektor penangkap microelektron (GC-μECD). Kajian lapangan telah dilakukan dalam persekitaran tropikal Malaysia untuk mengenal pasti corak penyebaran dan kekekalan racun lambda cyhalothrin di dalam ekosistem sawit. Keputusan daripada kajian ini menunjukkan peratus perolehan semula bagi lambda cyhalothrin masing-masing berada di dalam julat 87-90%, 96-109%, 81-105% dan 83-90%, untuk sampel minyak sawit mentah dan minyak isirong sawit, daun, tanah dan air pada tiga tahap kepekatan. Had kuantifikasi bagi kaedah analisis ini ialah 0.02 mg/kg manakala had pengesanan bagi kaedah ini ialah 0.002 mg/kg. Kajian ini telah pun dilakukan di ladang Amcorp bertempat di Sepang, Selangor dengan tiga plot experimen iaitu plot kawalan, plot dos saranan dan plot dua kali ganda dos saranan. Setiap dos kepekatan dilakukan sebanyak tiga replikat untuk setiap plot yang terlibat. Racun lambda cyhalothrin (ALERT 2.8 EC) disembur kepada daun kelapa sawit mengikut dos saranan dan dua kali ganda dos saranan pada 31.5 and 63 g a.i./ha. Kesemua sampel (minyak sawit mentah dan isirong sawit, tanah, daun, air) diambil pada hari yang sama bagi memudahkan pensampelan dibuat iaitu pada hari 0 (6 jam selepas rawatan), 1, 3, 5, 7, 9, 10, 12, 14, 16, 18, 20 dan 29 hari selepas rawatan. Kepekatan awal yang didapati pada hari 0 (6 jam selepas rawatan) ialah 0.47 and 0.83 mg/kg mengikut dos saranan dan dua kali ganda dos saranan. Racun lambda cyhalothrin tidak lagi dikesan selepas hari ke 14 dan 18 mengikut dos saranan dan dua kali ganda dos saranan. Tiada racun lambda cyhalothrin yang dikesan pada tiga sampel yang lain iaitu minyak sawit mentah dan isirong sawit, tanah dan air. Tempoh setengah hayat ($t_{1/2}$) bagi lambda cyhalothrin di dapati pada 3.96 dan 4.88 hari mengikut dos saranan dan dua kali ganda dos saranan bagi sampel daun sawit.

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Table of Content

List of Figures

List of tables

List of Symbols and Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic
AOAC	Association of Official Analytical Chemists
ADI	Acceptable Daily Intake
APVMA	Australian Pesticide & Veterinary Medicine Authority
BDL	Below Detection Limit
CPO	Crude Palm Oil
CPKO	Crude Palm Kernel Oil
CS	Capsule Suspension
DDT	Dichlorodiphenyltrichloroethane
DT50	Disappearance Time (50 %)
EPA	US Environmental Protection Agency
EC	Emulsifiable Concentrates
ECD	Electron Capture Detector
EU	European Union
FDA	U.S. Food and Drug Administration
FENP	Fenpropathrin
GC	Gas Chromatography
GCB	Graphitized Carbon Black
Ha	Hectare
HCH	Hexachlorocyclohexane
HCN	Hydrogen Cyanide
ICH	International Conference on Harmonization
IPCS	Internationa Programme on Chemical Safety
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
*K*ow	Octanol Water Partition Coefficient
*K*oc	Water–Soil Organic Carbon Partition Coefficient
FDA	Food and Drug Administration
LD50	Lethal Dose (50 %)
LOD	Limit Of Detection
LOQ	Limit Of Quantitation
MHE	1- Methylheptyl Ester
MPI	Maximum Permissible Intake
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council
MS	Mass Spectrometry
Pa	Pascal
PHI	Pre Harvest Interval
PSA	Primary Secondary Amine
R&D	Research and Development
SC	Suspension Concentrates
S/N	Signal to Noise Ratio
SPE	Solid Phase Extraction
TOF	Time-Of-Flight
TIC	Total Ion Chromatogram
TMRC	Theoretical Maximum Residue Contribution

TRI	Toxic Relearse Inventory
ULV	Ultra Low Volume
USA	United States of America
USP	United States Pharmacopoeia
WHO	World Health Organizations
WP	Wettable Powders

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

In the current competitive world, Malaysia as a developing country needs to widen its economic resources to increase income and productivity of the country. One of the sectors that assists in constructing the nation is the agriculture sector, which generated RM 56, 095 million based on gross domestic product by economic activity in 2013 and estimated to produce RM 58, 245 million in 2014 (Kementerian Kewangan Malaysia, 2015). Agriculture has a pivotal role in the Malaysian economic due to its high demand by the local and international industries. Palm oil serves as one of the biggest commodity in Malaysia with the highest production based on commodities for the agricultural sectors in 2012 (MPIC, 2015).

History of palm oil plantation in Malaysia began from an experimental phase since the late 1800s to early 1900s. This experimental oil palm garden was planted by Federated Malay States government in 1903 at Batu Tiga, Selangor. This is followed by the development phase, beginning with palm oil plantation in Tennamaran estate from 1917 to about 1960. From this point onwards, palm oil grew and expanded with 55,000 hectares (ha) in 1960. In 1966, Malaysia was the biggest palm oil producer which overcomes other countries, including Indonesia, Nigeria and Zaire, to become the world largest palm oil exporter. By 1980, palm oil plantation exceeded millions of hectare producing 2.5 million tonnes of crude palm oil. Now, Malaysia is consistent in producing palm oil and its products with 5,076,929 ha in 2012 and based on preliminary data, 5, 393, 235 ha was utilised in 2014. Factors influencing the rapid and consistent growth of the Malaysian palm oil plantation are based on several reasons. As for the palm oil, the commodities itself, faster maturity, low labor requirement, fewer disease problem and more stable prices compared to rubber plantation enabled the industry to

expand rapidly. The suitable soil and climate, political stability, good infrastructures and government policy, incentives, research and development (R&D) activity were part of the reasons concerning the success of palm oil in Malaysian history.

In comparison with other oils and fats in the world, palm oil is the largest oil production in the world, with 55, 760, 000 tonnes in 2013, and soybean oil at 42, 821, 000 tonnes in the second position. Malaysia is one of the biggest contributor to this statistics, with 19, 215, 000 tonnes of palm oil in 2013, with Indonesia still leading at 28, 300, 000 tonnes of palm oil (MOSTA, 2014). Recognizing the impact and potential of palm oil in the world market and its potential in boosting the Malaysian economy, two government agencies were involved in researching, developing and marketing the Malaysian palm oil industry.The two agencies involved are Malaysian Palm Oil Board (MPOB) and Malaysian Palm Oil Council (MPOC). Malaysian Palm Oil Board (MPOB) focuses on improving, optimizing and expanding the use of oil palm and its products, while Malaysian Palm Oil Council (MPOC) functions as an agency that promotes Malaysian palm oil and its products within two major stages. Those stages are, to strengthen the image of palm oil and to develop better acceptance towards palm oil with various campaigns on advantages of economics, technologies and environmental sustainability.Therefore, in maintaining the sustainability of palm oil in the world market, research and development (R&D), as well as innovation in the palm oil industry need to be strengthen to ensure good quality of palm oil products can be produced in the future.

1.2 Pyrethroid Residues & Impact on Environment

The first modern pesticide is dichlorodiphenyltrichloroethane (DDT) was established in 1939 at Geigy, Switzerland by Paul Muller. Paul Muller was successful in creating this formulation that can be used in protecting human from diseases (malaria, etc) and was awarded a noble prize in 1948 (The History of Pesticides, 2008). Modern pesticide progresses with production of other pesticides and being categorized into specific group known as insecticide, herbicide and fungicide.

As demand for pesticide increases, scientist begin to realize the harmful impacts of pesticides towards the environment. Previous studies have proved the detection of dieldrin, endrin and aldrin in environment that are non-degradable and bioaccumulate in living organisms (Buser, 1995, Ritter *et al.,* 1995). DDT, the pioneer of modern pesticide was also found at United States. There are also cases of sudden uncontrolled dead fish in Mississipi that was due to the presence of aldrin in the rivers water after several investigations (Delaplane, 2000). Based on cases reported, United States followed by Europe banned the use of organochlorine insecticides beginning from 1972, due to high environmental persistence and bioaccumulation of these organochlorine pesticides residues in the environment.

Now, approximately 1600 pesticides are used worldwide, with more than 100 chemical classes for food production (Macbean, 2012). Thus, with more pesticides residues present in the environment, it is more likely to pollute the environment and emit negative impact, not only to human but also to the flora and fauna present in the nature. Therefore, the usage of pesticides and other chemicals in plantation need to be controlled and minimized to avoid harmful effect of man made chemicals towards the environment and to ensure that pesticide residues are not detected in the palm oil and its products.

Lambda cyhalothrin, a pesticide that belongs to the pyrethroid group is commonly known as man made chemical which are used in nature. Pyrethroid as detected in the environment may originate from various places, depending on the purpose of its use. One of the main uses of pyrethroid is for agricultural purpose that is applied to the crops by various means, either by using planes, helicopters, trucks, tractors, or hand-held applicators. For indoors application, pyrethroid is applied through aerosol bombs and sprays, commonly used in controlling flying insects like mosquitos and flies. There might be cases of indirect introduction of lambda cyhalothrin in the environment, for example, during the production process, whereby, some of this compound may be introduced to the environment due to human mistake.

The most important route of pyrethroid exposure towards the general public will be by direct ingestion of foods, from insecticides sprayed to vegetables and fruits. Public may also be exposed to the pyrethroid, through daily household activities including use of mosquito repellents, pet shampoos, lice treatments and household sprays (ATSDR, 2003).

1.2.1 Fate of Pyrethroid in the Air

Emission through air will be the first possible pathway of pyrethroid to the environments based on usual practice of spraying insecticide by covered aerial, ground and indoors spraying. There are several pyrethroid groups of chemicals that are listed as Toxics Release Inventory (TRI) by EPA, which requires the release of these chemicals to be reported to the EPA (EPA, 1995). These pyrethroids include allethrin, cyhalothrin, bifenthrin, permethrin, cyfluthrin, fenpropathrin, resmethrin, fluvalinate, phenothrin, and tetramethrin (TRI99, 2001). Most of pyrethroids are known to degrade faster in the present of sunlight or through its reaction with other compounds in the atmosphere. Pyrethroids are reported to be only present in the air for one or two days undergoing

degradation. Usually, rain and snow remove the pyrethroids from air that are not rapidly degraded (ATSDR, 2003).

1.2.2 Fate of Pyrethroid in Water and Aquatic System

Properties of pyrethroid that are strongly hydrophobic, influence the behaviour of pyrethroid in water and aquatic system. Pyrethroid compounds with log K_{ow} ranging from 4 to 7.6, contain a water soluble properties that are quickly reduced in the aquatic system (Oros & Werner, 2005). Research on spray drift of lambda cyhalothrin shows that lambda cyhalothrin degrades faster from the water column (half life of approximately one day). Thus, concluding that, pyrethroid will only cause a short-term community stress within water exposure (Arts *et al.,* 2006).

The main sources of pyrethroid in a water system are from runoff and rainfall originally from agricultural fields. They are not sprayed directly to the water system as most of pyrethroid are extremely toxic to fish. Potential sources of pyrethroid are from spray drift that come from nearby water sources. Strict guidelines regarding pyrethroid usage in water system must be adhered, for examples permethrin, phenothrin and resmethrin, that are frequently used in mosquito control, are banned to be used in open water or within 100 feet of lakes, rivers, and streams based on their higher toxicity to fish (EPA, 2000).

Lambda cyhalothrin behaviour in water is shown by rapid dissipation based on several studies. For example, lambda cyhalothrin was applied to mesotrophic and eutrophic ditch microcosms three times, for a week, at different concentrations of 10, 25, 50, 100, and 250 µg/L. Results show rapid dissipation from the two systems, with only 30% of the amount applied remaining in the water phase (Roessink *et al.,* 2005). When lambda cyhalothrin was applied to a laboratory-simulated rice paddy water, fate of both gamma- and lambda-cyhalothrin were found to be rapidly loss from water

system, with no gamma or lambda cyhalothrin detected after 3 to 4 days, respectively (Wang *et al.* 2007).

The short presistence of pyrethroid in water is noted in aquatic system.Usually, pyrethroid will be transported within aquatic system due to absorption by fine particulates (Gan *et al.,* 2005). This is proved by the half life of pyrethroid in water column that are only between days to weeks, compared to adsorbed pyrethroid in particulates that are reported to be between 150 to 200 days for half life of pyrethroid in sediments (Amweg *et al.,* 2005).

Based on the location of pyrethroid, more than 97 % of the total mass of pyrethroid were on the suspended solids and in stream water. About 10% to 27% were found as a freely dissolved phase (Liu *et al.,* 2004). Based on previous studies on bifenthrin and lambda cyhalothrin, these two pesticides penetrate through ditches by retention and adsorption. Initial runoff concentrations of both pesticides were found to be 666 µg/L (bifenthrin) and 374 µg/L (lambda-cyhalothrin). These figures were reduced to 7.24 and 5.23 µg/L, respectively, moving to 200 m downstream. Nevertheless, no pyrethroid residues were detected at 400 m downstream (Bennett *et al.,* 2005).

Normally, pyrethroid concentration in sediment will increase along a drainage channel downstream, as moving far from initial sedimentation pond. This is shown by previous studies, for example, study on bifenthrin sediment with initial concentrations in the pond at an averaged of 0.33 mg/kg, increased to 2.27 mg/kg at 104 m downstream, and 10.64 mg/kg at 145 m downstream. Similar trending was observed to *cis*-permethrin, with initial sedimentation pond concentrations of 0.77 mg/kg that

increased to 1.10 and 4.45 mg/kg at 104 and 145 m downstream, respectively (Gan *et al.,* 2005). This was later concluded to be caused by the increasing organic carbon and clay content of the sediment as moving from the sedimentation pond. As mentioned previously, this is due to the behaviour of pyrethroids that strictly adhere to fine organic particulates. Therefore, the pyrethroid-bound fines are likely to be transported by downstream flow, resulting in downstream enrichment (Katherine *et al.,* 2012).

1.2.3 Fate of Pyrethroid in Soil

Pyrethroid behaviour in the soil environment is dependent on the properties of pyrethroid group chemicals. Pyrethroid normally will bind to the dirt and is less labile in soil. For example, fraction of fenvalerate was found to bound greatly in silty clay loam soil as compared to sandy loam soil (Lee, 1985). Pyrethroid is not easily absorbed by the roots of plants and vegetation due to its ability to bind strongly to the soil. Thus, pyrethroid normally will not leach into groundwater and it does not contaminate drinking water supplies and volatilises slowly from soil surfaces. Degradation of pyrethroids is mostly by microorganisms in the soil and by the action of sunlight on the surface of the soil. Recent studies show that some microbes are able to degrade pyrethroids by esterase production (Thatheyus & Selvam, 2013).

As most of pyrethroids have relatively low vapor pressures and Henry's law constants, the volatilization of pyrethroids from soil is to occur slowly (ASTDR, 2003). For example, half life of pyrethroids in aerobic soil condition (e.g., expected field conditions) is varied with half life ranging from 11.5 days for cyfluthrin to 96.3 days for bifenthrin (Oros & Werner, 2005). Photolysis is an important degradation pathway for pyrethroids in the soil and depends on the soil characteristics. A study on esfenvalerate indicated the increasing half life of pyrethroid in different soil systems under dark conditions, with a comparison between half life of 7.8 to 100.0 days under continuous irradiation and half life of 150.0 to 553.4 days in the dark (Katagi, 1991).

The present of plant canopy also influences the concentration of pyrethroid in the soil, previous study indicated the decreasing concentration of cypemethrin in the agricultural field soils under crop covers, with approximately one-tenth concentration from those in the bare soil following a spray event (Wiles & Jepson, 1994).

1.3 Research Objectives

The objectives of this research are:

1.1 To determine lambda cyhalothrin residues in crude palm oil and crude palm kernel oil and in oil palm plantation soil, leaf and water.

1.2 To determine the half life of lambda cyhalothrin residues in crude palm oil and crude palm kernel oil and in oil palm plantation soil, leaf and water.

1.3 To evaluate the field persistence of lambda cyhalothrin in palm oil, leaves, soil and water.

CHAPTER 2: LITERATURE REVIEW

2.1 Malaysia Oil Palm Industries

Based on planted area, oil palm remains as the biggest commodity being planted in Malaysia with 5, 076, 929 Ha, followed by other commodities such as rubber (1, 041, 187 Ha), cocoa (11, 748 Ha), pepper (14, 791 ha) and tobacco (2, 526 Ha) in 2012 (MPIC, 2015). This indicates the huge role and impact of palm oil industry towards Malaysian agriculture sector.

In 2013, Malaysia exported palm oil and its products with a gross value of RM 63,147.11 million and this value increased to RM 66, 123.88 million in 2014. Malaysia exported palm oil and its products to various parts of the world. This includes 150 countries throughout the world, including China, Europe, India, Pakistan, United States, Ukraine, Japan and Egypt (Wahid, 2009).

Product exportation from palm oil based includes palm oil, palm kernel oil, palm based products and palm kernel cake (MPIC, 2015).The two main products which are the crude palm oil and crude palm kernel oil are obtained from the mesocarp of palm oil fruit and kernel of palm fruit respectively. Palm oil and palm kernel oil differ in term of nutrition and its chemical properties. Based on the chemical components, crude palm oil consists of 45 % palmitic acid, 40 % oleic acid and 10 % linoleic acid. For crude palm kernel oil, lauric acid cover 48 % of its chemical components. There are several minor components in palm oil, yet provide significant contribution to our health. They include phospholipid, sterol, tocopherol, tocotrienol, carotene, triterpene alcohol, squalene, aliphatic alcohol and aliphatic hydrocarbon. This minor component only presents about 1 % in crude palm oil and 0.8 % in crude palm kernel oil (Choo Yuen May, 2015).

2.2 Pesticide

According to EPA (2015) pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.), including insecticide, herbicide, fungicide, and various other substances used to control pests. Meanwhile, Pesticide Act 1974, stated that pesticide is a substance that contains an active ingredient or any preparation, mixture or material that contain any one or more of the active ingredients as one of its constituents. The first research on the consequences of pesticide were conducted by Tennessee Valley Authority in the late 1930s and early 1940s. Their study were intended to look on the non-target effects of oil and Paris Green used on mosquito vectors of malaria (Tazwell, 1978). A paper by Goodnight (1942) on toxicity of sodium pentachlorophenate and penthachlorophenol on fishes were said to be the first published paper on modern pesticides (Goodnight, 1942).

Historically, the development of pesticides can be divided into three phases according to Dhaliwal *et al.* (2004). These phases were determined by the nature of the pesticide itself. It began with the era of natural pesticides which were used in ancient Greece before 1870s including the application of sulphur. Second phase was known as inorganic synthetic pesticide era, as pesticides at this time were made from natural and inorganic materials. This era began in 1870s until 1945. The third phase was in 1945 with the introduction of organic synthetic pesticides. Amongst this group of pesticides are 2,4 dichlorophenoxyacetic acid, DDT, HCH and dieldrin etc. The use of organic synthetic pesticides has significantly changed the agricultural sector as a whole. This is due to the contribution of these pesticides that secure and enhance the agricultural production as the growing use of organic synthetic pesticides at that time. In earlier days, three types of organic synthetic insecticide were commonly used, that are carbamate, organaphosphorus and organochlorines pesticides. Later on, insecticide use

declined gradually, with the introduction of herbicides and fungicides. The consumption of herbicides is dominant, and based on the trends worldwide (Xu, 1997). Europe is the largest consumer of pesticide, followed by Asia, whereby the use is mostly on fruit and vegetable crops (Zhang, 2011).

2.3 Problems and Pesticide Application in the Oil Palm Plantation

2.3.1 Herbicides & Weeds

In oil palm plantation, weeds are always regarded as a major problem. Weeds are defined as any plants that grow at undesirable places and time (Shari, 2012). Weeds comprises mixture of grasses, sedges and broadleaves that depend on the surrounding's climatic and environment to produce specific types of weeds. As for palm, its growth creates a shade or canopy to the weeds, and subsequently determines the nature of the weed compositions. Usually, grass type of weeds are dominant species, as palms continue to grow (Wan Mohamed, 1987).

In plantation, weeds become the competitors to the palms as they compete for nutrient, space, water supply and light intensity. But, some types of weeds can be used to prevent soil erosion in the plantation. In Malaysia, the most common leguminous cover crops in oil palm and also rubber are *Mucuna bracteata, Pueraria javanica, Calapogonium caeruleum, Calapogonium mucunoides* and *Centrosema pubescens*. Weeds also have a role in carbon and nitrogen fixing, and this support a sustainable ecosystem within the plantation (Kobayashi *et al.,* 2003; Goh *et al.,* 2007; Goh *et al.*, 2014, Shari, 2012). Eventhough weeds have its own beneficial contribution to the ecosystem, the population in the plantation need to be controlled. This will avoid damage, yield loss and reduction of income (CAB International, 2011).

They are several practises of managing weeds. These include mechanical weeding, flooding, covering with organic or inorganic materials (e.g. mulch or plastic sheeting), slashing, hand weeding and treatment with chemical or biological substances

(herbicides) (CAB International, 2011). Using chemical substances known as herbicides are established method of controling weeds. Herbicides are effective, reliable and economically savvy, therefore, act as an efficient tool of weeds management in plantation (Estorninos & Moody, 1988). Each type of herbicide works differently depending on their specific mechanism of action towards weeds (Sani *et al*., 1991; Hoerlein, 1994). Supposedly, the effect of herbicide treatment towards different type of weeds are the same. But, this does not happen as different herbicide reacts differently towards different type of weeds. This is shown by the change in the compositions of weeds due to the selectiveness of herbicides (Wrucke & Arnold, 1985, Swanton *et al*., 1993). This informally provides awareness on the importance to be selective in choosing the right herbicide for different type of weeds.

In oil palm plantation, herbicides are used for several purposes. This includes clearing for replanting of palms, ground cover management, clearing for path, roads and waterways, conserving the free weeds area in circular palm base and treating volunteer oil palm and epiphytes (CAB International, 2011). In Malaysian palm oil industry, herbicides use was estimated at RM 334 million in 2009 and became the most used pesticide in Malaysia (Malaysia Agribusiness Directory, 2010). Four types of herbicide are commonly used in Malaysian palm oil plantation. They are glyphosate, glufosinate ammonium, metsulfuron methyl and paraquat (Ashton & Crafts, 1981; CAB International, 2011; Collins, 1991; Turner & Gillbanks, 2003).

2.3.2 Insecticides, Pests & Diseases

One of the problems associated with any plantation including oil palm plantation is pest. Thus, researchers continuously study and identify the mechanisms to control and eliminate harmful pest in the plantation. Table 2.1 listed the diseases and pests related to the palm oil plantation based on the different stages of the palm.

Table 2.1: Pest and diseases of palm oil plantation (Ainie *et al.,* 2007)

Stage	Diseases, disorders and pests
Nursery	Anthracnose, leaf spot disease, aphids, cockchafers
Stem and root	*Ganoderma* trunk rot/basal, stem rot, upper stem rot, termites
Bunch and fruit	Bunch and fruit rot, *Marasmius palmivorus*, bunch moth (*Tirathaba rufiwena*)
Young palms	Grasshoppers, rhinoceros beetle
Mature palms	Beetles, termites
Fruits	Rat (*Ratticus tiomanicus*), birds (*Psittacula longicanda, Psittinus cynurus* and *Loriculus galgulus*)
Mature leaf	Crown diseases, white stripe, patch yellow, leaf eating caterpillars, bagworms, nettle caterpillars

Insecticides are a group of substances specifically created to kill unwanted insects in the environment with its known heterogenous toxicity. As it is explicitly created, the mode of action of insecticide is to be toxic to insect and less toxic to other non targerted organisms such as plant and human. Insecticides are seldomly used in oil palm plantation (Timothy, 2012). Basically, insecticide are only being used when there are infestation of insects, that is beyond the workers control (Ainie *et al.,* 2007). In the oil palm plantation, two common groups of pesticides were used, namely organophosphorus compounds and synthetic pyrethroids.

Both organophosphorus compounds and synthetic pyrethroids are frequently used to control and manage the spreading of rhinoceros bettle and bagworms. Bettles are well known pests in oil palm plantations.Various types of bettle can be found in the plantation depending on the region where the palms are planted. For example, *Rhynchophorus palmarum* and *Alurnus humeralis* are two types of beetle that are harmful in America, *Oryctes monoceros* in West Africa and *Oryctes rhinoceros* in South East Asia (Cock *et al.,* 1987).

As stated in Table 2.1, in South East Asia, from the Malaysian perspective, *O. rhinoceros* attacking method is based on the young palms.To identify whether *O. rhinoceros* attack provides either small or big impact towards plantation, this depends on the replanting method applied in each plantation, every one or two years. Generally, the first replanting phase encounters less impact of damages compared to second replanting phase. This is due to the time frame for the beetles to mutliply and grow bigger in terms of their population compared to the second phase of replanting. In the same phase, there may be two peaks of damages, where the second peak are usually higher and destructive compared to the first peak (Noor Hisham *et al.,* 2013).

Lost from bettle attacks may vary depending on the intensity of the attacks towards plantation. About 15% of the area may be lost from severe damage, thus yielding loss up to 25% (Liau & Ahmad, 1991; Samsudin *et al.,* 1993). This figure can increase up to 30- 40% with a single defoliation about 50% over a two year period (Wood *et al.,* 1973). There are several reported damages by *O. rhinoceros* in oil palm plantation. It is stated that once happened in Samoa, where the outbreaks of this pest caused a decrease to about 60% in oil palm yield on the first year of harvesting, that resulted to a change for coconut cultivation from oil palm (Dhileepan, 1994, Kalidas & Rethinam, 1998 & Mariau, 1999). In Malaysia, the establishment of *O. Rhinoceros* come from the emergence of coconut cultivation. Beetles introduction to Malaysia from other countries through several activities including shipping and cargo transportation of timber, nursery trade and transportation of habitat material. *O. Rhinoceros* soon adapted well on coconut trees which were abundant along the Malaysia coastline through a range of host. This later led to establishment of *O. Rhinoceros* population along the east and west coast of Malaysia (Gurmit, 1987). In Malaysian palm oil plantation, it is reported that damage done by *O. Rhinoceros* could led to average crop loss of 40 % to

92 % during the first year of harvesting and 15 % reduction in canopy size (Chung, 1999).

The adult beetle will bore young fronds and cause fracture to the frond, as it feeds on the soft tissue. The palms might be killed if its meristem tip damages. They can stay on the tunnel for about one week before migrating to another places to find other food sources. The lifecycle of an adult beetle ranges from 2 to 7 months, therefore, towards its lifetime, it may damages several palms (AAR, 2010). Damage caused by beetles also provide an entry point for red palm weevil *(Rhynchophorus ferrugineus)* and other fungal pathogens, that leads to bud rot disease.

Prior to applying insecticides to the plantation, there are several ways of handling *O. rhinoceros.* This biological strategies are either by applying entomophagus fungus *Metarhizium anisopliae* or oryctes virus (Ramle *et al.,* 2005). Both techniques are specific towards the beetles, effective in controlling, but does not totally remove this pest in the affected area. It also create endemic to the population and is capable of generating infection above 75% from the total population.

Insecticides also help in controlling the outbreaks of bagworm. Bagworms belong to Psycidae family, and the common species that are present in oil palm plantation are *Metisa plana, Mahasena corbetti* and *Pteroma pendula* (AAR, 2010). Bagworms depend on the presence of shelter and food sources (nectar) supported by benificial plants in the oil palm ecosystem. It is reported that in India, the second outbreak of bagworm after insecticide application and this leaf web worm caused a loss of 3.88 % photosynthetic area per palm by means of defoliation (ICAR, 2012). As for Malaysia, repeated outbreaks of *M. plana* in southern Perak are a major problem for oil palm plantation. Yield declining around 30-40 % over 2 years may occur due to decrease in photosynthesis rate by a moderate defoliation (10-13%). A moderate

defoliation (10-13%) can cause a yield to decline around 30-40% over 2 years due to decrease in rate of photosynthesis.

In handling the spreading of bagworms, several parasitoids were reported to feed on bagworms in Malaysia. *Apanteles* sp., *Xanthopimpla* sp., *Pediobius* sp., *Brachymeria* sp. and a non-occluded virus on larvae are amongst those parasitoid involved in controlling the population of bagworms in plantation (Basri *et al.,* 1995, Sankaran & Syed, 1972). This parasitoid are attached to some plants, indicating the need to keep flourishing those plants involved. They include *Cassia cobanensis, Crotalaria usaramoensis, Antigonan leptopus* and *Euphorbia heterophylla* (Sankaran & Syed, 1972).

Both *O. Rhinoceros and* bagworms are also known to be control by biological way through the used of *Bacillus thuringiensis*. There are several advantages of choosing this technique in controlling the pests in plantation that includes low mammalian and non target impacts and fast breakdown towards uv lights (Whalon & Wingerd, 2003). *Bacillus thuringiensis* also helped in dealing with the spreading of pest in plantation when resistance to chemical agent becoming a serious problems in plantations. The mechanism action of this biological technique is by the ingestion of plant material through the foregut where the ingested material are broken down into small pieces. This plant material will undergo initial enzymatic digestion in ectoperitrophic space that are located in organisms midgut. This digested material will undergo further digestion and being absorbed by the microvilli covered cells of the midgut epithelium with finally kills the target organism (Gringorten, 2001).

Therefore, as stated earlier, insecticide will be applied when the population of insects, especially rhinoceros bettle and bagworm population are not under control anymore. Commonly applied insecticide of organophosphorus compounds are

monocrotophos, methamidophos, chlorpyrifos and acephate, while for synthetic pyrethroids are cypermethrin, lambda cyhalothrin and deltamethrin.

2.3.3 Fungicides Infections & Diseases

Fungicides functions as a destroyer or inhibitor to the growth of fungi. Various economic sectors (industry, agriculture, home and garden) use fungicides for many purpose. These include crops protection, seedlings, storage, as a slime controller in paper industry, fabrics and carpets protection and mold suppression (William M. Simpson, 2006).

In the oil palm plantation, four type of fungicides are commonly used which are benomyl, thiram, captan and hexaconozole. Benomyl is specifically used against various fungal diseases in plantation. Carbendazim, the degradation product of benomyl is the source of reference in detection of benomyl in agroenvironment samples (EXTOXNET, 2015). While, thiram is used in preventing damaging of crop in the field, avoiding spoil in the crop, either while in storage or transport. Captan is also one of fungicide used for diseases protection from fungi. Beside that, captan is also involved in seed treatment to protect the young plant from being rot and damping off. Toxicology test upon captan shows negative results in any tetragenic, carcinogenic or mutagenic effect, thus enabling captan to improve the final fruit product, by providing healthier and brighter colour appearance to the fruit (EXTOXNET, 2015; FDA, 1967).

Hexaconozole is a systemic fungicide used to treat and control *ganoderma* in oil palm plantation. In plantation, *ganoderma* can be found in three sources which include inoculum left from the alternative host plants, inoculum from infected oil palm (spreading by root contact) and the airborne basidiospores (produced and released by fruiting bodies or sporophores) (Chung, 2011).

There are different kinds of ganoderma species but the most common species found in Malaysia is *Ganoderma boninense.* Many studies identified basal stem rot as a the disease caused by *Ganoderma boninense* and it is a major and serious disease in the oil palm plantation in Malaysia, Indonesia and some estates in south east asia (Ariffin *et al*., 2000; Idris *et al.,* 2000a, b; McGuire *et al.,* 2015; Singh, 1991; Susanto *et al.,* 2005*,* 2009; Turner, 1981). Basal stem rot is a disease caused by the fungal pathogen that kills the roots and affects basal stem. This disease stops the movement of water and nutrient to the upper part of the palms, especially towards the foliage (Chung, 2011).

Ganoderma attack can occur at the early stage of planting in between 12 to 24 months old palm (Ariffin *et al.,* 1996). But, probability of basal stem rot disease to occur in immature palm is low and occurs randomly. Based on observation, this disease frequently happens in oil palm planted in ex-coconut planting compared to plantation at ex-rubber plantings. Several symptoms were detected in palms that are infected with this disease. Infected palm may show symptoms like, unopened new fronds (spears), wilting of green fronds (hanging downwards like a 'skirt'), yellowing of fronds (due to nutrient deficiency) and producing a smaller canopy due to smaller fronds (Chung, 2011).

Impacts caused by *ganoderma* in plantation should be taken seriously. After 15 years since the first detection of *ganoderma* in palm oil plantation, it is reported that, it can kill as many as 80% of oil palm (USDA, 2012). When the basal stem rot disease reaches epidermic situtation in coastal estates, it simultaneously cause the dead palms to reach until 85 % from the whole populations, as they reach 25 years old (Singh, 1991). This disease also spreading in Perak, with increasing cases from 5.4% to 44.1% in 14 years time (Khairudin, 1990). For every 31% to 67% basal stem rot disease reported, statistic shows a yield reduction of 26% to 46% (Singh, 1991).

Besides having hexaconozole as a treatment and control tool for *ganoderma* species, research continued to find the best way of handling this fungi. To improve in identification and localisation of this pathogen, molecular genetic and biochemical markers methods was applied (Bridge *et al.,* 2000). Futhermore, genome sequencing of several virulent and non virulent strains of *ganoderma,* helped to identify the gene related to virulence and help breeders to develop more tolerant varieties of oil palm. Technologies of molecular genetic is also able to diagnose this disease and its early stages, and preventing the spreading of *ganoderma* species (USDA, 2012).

2.4 Lambda Cyhalothrin

2.4.1 History and use of Synthetic Pyrethroid

The control of pest in its earlier days until 1940s, generally used natural sources, as the main ingredient, either by direct consumption or after simple extractions or treatments. They are known as the "first generation" insecticides that consists of inorganic arsenic- or fluorine-containing toxicants or botanicals such as nicotine, rotenone and pyrethrins. From the 1940s onwards, the first generation insecticides except pyrethrins, were displaced by the "second generation" insecticides or synthetic organic pesticides. The second generation pesticide provided a complete control of the pest, with high potency or persistence or either both properties at low cost. This group of insecticides consist of four classes known as organophosphorus, methycarbamates, chlorinated hydrocarbons and pyrethroids, with mode of action against the nerve system of the pest.

Pyrethroids are synthetic man made chemicals that are originally based from pyrethrum or pyrethrins. Pyrethrum is widely used in control of pest insects in the household, barns and livestock. The pyrethrum swiftly response towards houseflies, mosquitoes and other flying insects, causing these insects to die after a few minutes or hours later, with the right doses applied. Pyrethrum is modified in its final development

with addition of an additive or synergist that are discovered in 1949. Piperonyl butoxide in addition to pyrethrum increase the potency of pyrethrum, more economical and increase the potential of insect to die rather than recover. Pyrethrum was considered to be the safest insecticide and was once labelled as 'non toxic to humans and pets'. This labelling is not allowed as of now, but usage for more than a century, with only few cases related to pyrethrum, established this insecticide as one of the safest pesticide to human. Pyrethrum and its synergists are regulated under Environmental Protection Agency (EPA) and Food and Drug Administration (FDA). The maximum residual limit to various plant products are commonly 1 to 3 ppm for pyrethrins and 8 to 20 ppm for piperonyl butoxide (Casida, 1980).

Synthetic pyrethroid is developed from an extensive research by British, Japanese and French scientists, in finding a more stable and effective compounds with a mammalian toxicity that are low as compared to the model of Pyrethrin I (Katherine *et al.* 2012; Pap *et al.,* 1996). The first pyrethroid, allethrin, was synthesized in 1949 and commercially marketed in 1952 to be used as a control for household insects (Katherine *et al.,* 2012). The first set of pyrethroid compounds only show a moderately higher insecticidal property than pyrethrin I, but at this stage, it is proved that these are potent knockdown agents. The actions are through the voltage-sensitive sodium channels that affect the nerve impulse transmission in insects (Soderlund & Bloomquist, 1989).

Pyrethroid groups are further improved with production of tetramethrin in 1964, with increase in efficacy by changing the alcoholic component to 5-benzy-3-furylmethyl moiety. Resmethrin followed subsequently, whereby this compound was successfully synthesized in 1967 and marketed in 1969 to be used as household and public health insects control (WHO, 1989a). Both are the first synthetic compounds with good knockdown activity, lower mammalian toxicity and greater insecticidal activity than the natural esters (Katherine *et al.* 2012). Next, phenothrin was produced in 1969, with

advantages of being relatively cheap molecule and improved insecticide activity, which was commercialised in 1977, to be used in home, public health and protecting stored grain from insects (Pap *et al.,* 1996; WHO, 1990). The feature of photostability that enabled pyrethroid to be used in field was synthesized in 1973 with the introduction of permethrin. Marketing of permethrin began in 1977, and used in agricultural sector and also for mosquito nets, body lice and other household use (Pap *et al.,* 1996; WHO, 1990). Between 1976 and 1977, fenvalerate and deltamethrin were introduced to the market, mainly used for cotton, control of cattle insects and other crops.

Pyrethroid group further improved in term of its efficacy with the introduction of cypermethrin in 1974, used in agriculture, public health, household and animal husbandry (WHO, 1989b; Pap *et al,* 1996). Second generation pyrethroids (e.g., lambda cyhalothrin, bifenthrin, cyfluthrin) were available to the market in the 1980s (Weston *et al.,* 2004). With original patents expiring in the early 1990s, other manufacturers created a variety of new active ingredients by the mid-1990s (Pap *et al.,* 1996). Now, lambda cyhalothrin is widely used with various brand name products including Warrior, Scimitar, Karate, Demand, Icon, Reeva, and Matador (He *et al.,* 2008; Kumar *et al.,* 2010).

2.4.2 Chemical Class

Pyrethroids are known as synthetic pesticides originating from the modification of natural pyrethrin. In nature, there are six different pyrethrins that are divided into two different classes. Group one consists of crysantemic acid esters derivatives and group two comprises of pyrethric acid esters derivatives (Table 2.2) (Pérez-Fernández, García, & Marina, 2010).

Table 2.2: Pyrethrin group classes

Group 1	Group 2
Pyrethrin 1 ($C_{21}H_{28}0_3$) MW: 328.4 g/mol	Pyrethrin 2 ($C_{22}H_{28}0_5$) MW: 372.4 g/mol
Cinerin 1 ($C_{20}H_{28}0_3$) MW: 316.4 g/mol	Cinerin 2 ($C_{21}H_{28}0_5$) MW: 360.4 g/mol
Jasmoline 1 ($C_{21}H_{30}0_3$) MW: 330.4 g/mol	Jasmoline 2 ($C_{22}H_{30}0_5$) MW: 374.4 g/mol

Pyrethroid are specifically separated into two types that are based on differences in basic structures (Table 2.3). These differences are either due to the presence or absence of cyano group in alpha positions and the symptoms of poisoning (ATSDR, 2003). Type I pyrethroid are made up of an alcohol and esters of chrysanthemic acid, with furan ring, terminal side chain moieties and absence of a cyano moiety. Allethrin, the first pyrethroid produced in 1949, and other pyrethroids, such as phenothrin and permethrin, with the basic cyclopropane carboxylic ester structure are type I pyrethroids. The addition of cyano group increased the insecticidal activity of these synthetic pyrethroids to produce α-cyano type II pyrethroids such as deltamethrin, fenvalerate, cyfluthrin, cyhalothrin, and lambda-cyhalothrin. Terminal side chain of type II pyrethroids were be replaced by dichlorovinyl or dibromovinyl substitute and aromatic rings with 3-phenoxybenzyl alcohol derivatives in the alcohol moiety (Anadón *et al.,* 2013).

Table 2.3: Types of pyrethoid

Type I Pyrethroid	Type II Pyrethroid
Allethrin	Cyfluthrin
Bifenthrin	Cyhalothrin
Permethrin	Cypermethrin
Phenothrin(Bio)	Deltamethrin
Resmethrin	Fenvalerate
Tetramethrin	Fenpropathrin
Tefluthrin	Flucythrinate
	Fluvalinate
	Tralomethrin
	Flumethrin

2.4.3 Chemical Structure

Lambda-cyhalothrin is a 1:1 mixture of two stereoisomer that are (*R*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2 dimethylcyclo-propanecarboxylate and (*S*)-α-cyano-3- phenoxybenzyl (1*R*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate (Figure 2.1).

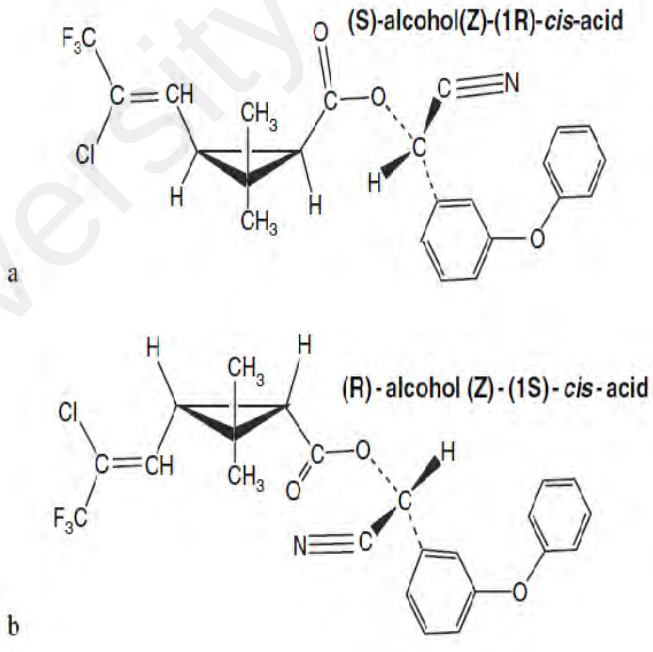

Figure 2.1: The chemical structure of two isomers of lambda cyhalothrin
a) (*S*)-α-cyano-3- phenoxybenzyl (1*R*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate
b) (*R*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2 dimethylcyclopropanecarboxylate
(He, L.-M., Troiano, Wang & Goh, 2008)

2.4.4 Physical and Chemical Properties

Lambda cyhalothrin in its solid form appears colourless at room temperature and yellowish in solution. Based on its physical properties (Table 2.4), lambda cyhalothrin has a low vapor pressure and Henry's law constant, indicating lambda cyhalothrin is not easily volatilized into the atmosphere. Its octanol–water partition coefficient (K_{ow}) value of 7.0, indicating high K_{ow} value which enable lambda cyhalothrin to penetrate lipid. This is due to higher potential of bioconcentrate for high K_{ow} pesticide. The water–soil organic carbon partition coefficient (K_{oc}) value shows the affinity of pesticide towards organic matter. The log K_{oc} of 5.20 display high K_{oc} value, thus higher affinity towards organic matter. This shows the low possibility of lambda cyhalothrin to contaminate groundwater due to unability to leach as dissolved residues in percolating water. But, this also indicates the primary sources of lambda cyhalothrin in aquatic system through sediments. Lambda cyhalothrin has a high tendency to adsorb to suspended particulate materials in the water column, including clay particles and organic matter. This provide a greater risk to a nontarget aquatic organisms. In addition, adsorbed molecules generally are less viable to sunlight and mircoorganisms, thus, indicating a decrease in rates of degradation compared to molecules that dissolve in the water column (Schwarzenbach *et al.,* 1993). Lambda cyhalothrin adsorption to sediments and suspended solid may help in reducing its acute toxicity towards aquatic organisms in a way that it reduces the short-term bioavailability of lambda cyhalothrin in the water column (He *et al.,* 2008).

Table 2.4: Chemical properties of lambda cyhalothrin
(He *et al.,* 2008; Kumar *et al.,* 2010)

Properties	Lambda Cyhalothrin
Molecular Weight	449.9 g/mol
pH	6.0 to 8.0.
Melting point/range	49.2°C (99.0% purity). 47.5 to 48.5°C (purity 96.5%)
Boiling point	187-190°C (at 0.2 mmHg)
Solubility in water	4×10^{-3} mg/L at pH 5.0 5×10^{-3} mg/L at pH 6.5 4×10^{-3} mg/L at pH 9.2 (purity 96.5%)
Octanol/water partition coefficient	Log K_{ow} = 7.0. (purity 99.0%)
log K_{OC}	5.20
Vapour pressure (Pa)	2×10^{-7} Pa at 20°C
Henry's law constant ($Pa.m^3/mol$)	0.02
Density (g/ml at 25°C)	416.3
Boiling Point (°C at 0.2 mmHg)	220
Solubility in other solvents (g/L)	450 (xylene)
Hydrolysis half life (day) pH 5 pH 7 pH 9	 Stable Stable 8.66
Photolysis half life (day) Water at pH 5 and 25°C Soil	 24.5 53.7
Bioconcentration factor (BCF) (fish)	2240
Soil adsorption K_{oc} (cm^3/g)	247000-330000
Soil degradation half life (day) Aerobic soil	 42.6
Aquatic degradation half life (day) Aerobic aquatic	 21.9

In hydrolysis study, lambda cyhalothrin is stable to hydrolysis at pH 5 and it slowly hydrolyses at pH 7 and rapidly hydrolyses at pH 9, based on study at the acid

moiety, over a period of 30 days at 25°C. The cyclopropane acid is a major product of hydrolysis at both pH 7 and 9 (2% produced at pH 7 and 73% at pH 9). Whereby, study on alcohol moiety revealed slightly different results with hydrolysis occurred very slowly at pH 4, slowly at pH 7 and fairly rapidly at pH 9, over a period up to 29 days at 25°C. In this study, 3-phenoxybenzaldehyde and 3-phenoxybenzoic acid were formed in all pH values, with 3-phenoxybenzaldehyde being the major compound formed at pH 9 (up to 78 % of the applied radioactivity) (FAO, 2013). This indicates the major degradation products of lambda cyhalothrin is 3-phenoxybenzaldehyde.

2.4.5 Mode of action

Pyrethroid mode of action is either to kill the targeted pest or distrupting their growth or development. Most of commercially available insecticides in the market targets the nervous system of insects, either at the synapse or the axon (Casida, 1980; Tsipi *et al,*. 2015). The pyrethroid acts is by binding to the protein in the nerve fibre that regulates the voltage-gate sodium channel. This gate functions as a door in allowing the stimulation of the nerve by gate opening and terminating the nerve signal by gate closing. Ion entering through this pathways will proceed to axon and cause excitation. If this pathways are left open, nerve cells will produce repetitive discharges and eventually causing paralysis (Bradbury & Coats, 1989; Shafer & Meyer, 2004).

Pyrethroid in its mechanism of action will bind to this gate and prevent it from closing normally. This will result in non stop simulation and tremors towards the targeted pest. Pest will lose control of their nervous system and are unable to produce a coordinated movement. Based on the type of pyrethroid, type I (e.g. permethrin) pyrethroid mainly interacts with sodium channel in the central nervous system.While, type II pyrethroids (e.g., lambda-cyhalothrin), interact with chloride and calcium channels that also involved in ensuring proper nerve function (Burr & Ray, 2004).

Chloride channels function in controlling the cell excitability. This conduits can be found in the brain, nerve, salivary gland and muscle that are regulated by protein kinase C. Chloride channels have variety of different functional types, but pyrethroid-sensitive channels are under maxi chloride channels class (Franciolini & Petris, 1990). Pyrethroid will decrease the maxi chloride channel current, which provide excitability, thus synergize pyrethroid actions on the sodium channel (Anadón *et al.,* 2013).

Type II pyrethroids also interfere with the GABA-gated chloride channels at high concentrations that may result in seizures, which usually happened in severe type II poisoning (Bloomquist *et al.,* 1986). This will only happen at higher dosage that affect the sodium flux. Type II pyrethroid interaction with ion channels is still unknown, but Type II pyrethroid will stimulate protein kinase C-dependent protein phosphorylation in vitro by a direct mechanism (Enan & Matsumura, 1993). As both sodium and chloride ion channel activities are regulated by phosphorylation state, this can be an important mechanism of action, despite the fact that pyrethroids are also capable of acting directly with the channels without any phosphorylation capacity at higher concentrations (Forshaw *et al.,* 1993).

Lambda cyhalothrin which is strong lipophilic nature enable biological membranes and tissues to readily absorb to them. In general, lambda cyhalothrin will penetrate the insect cuticle and interupt the nerve conduction, through opening channels within minutes, that leads to unability of feeding, loss of muscular control, paralysis, and eventual death. This is added with strong repellent effect of lambda cyhalothrin toward insects that make the knockdown mechanism much stronger (He *et al.,* 2008).

2.4.6 Toxicological profile

Pyrethroid toxicity differ from one to another, based on the various LD50 values (concentrations or doses that result in 50% mortality in exposed laboratory animals) of different pyrethroid chemicals group. These differences are based on variety of reasons including specific pyrethroid groups, ratio of stereo and optical isomers and vehicle (ATSDR, 2003). As a factor of stereochemistry, toxicity depends on the configuration of the chiral carbon adjacent to the carboxylic group (Pérez *et al.,* 2010). In comparison of type II and type I pyrethroids, it is found that type I pyrethroids have bigger degree of toxicity compared to type II pyrethroid, based on a much lower acute oral LD50 values of Type II than Type I pyrethroids.

In terms of R-, S- configuration, isomers of 1R conformation are more toxic that 1S conformation for type I pyrethroid (Narahashi, 1986). In contrary, for type II pyrethroid, S conformation on alpha carbon adjacent to the cyano group are more toxic than their R conformation. Thus, in determining the toxicity of pyrethroid group chemicals, major factors in determining the toxicity are their chemical properties and their exposure scenario in environments. When dealing with human exposure towards pyrethroid, this may involve other elements like impurities in technical grade pyrethroids to dispersal agents, wetting agents, solubility agents, and additional pesticides in a given end-formulated product (ATSDR, 2003). This is a summary towards the toxicological behaviour of lambda cyhalothrin by WHO through International Programme on Chemical Safety (IPCS). WHO/IPCS have classified lambda cyhalothrin as 'Moderately Hazardaous' (Class II) chemical, based on acute oral toxicity data (WHO, 2010). Lambda cyhalothrin can be classified as harmful, causing irritation to skin, eyes and upper respiratory system. Direct ingestion of lambda cyhalothrin could lead to neurological symptoms, like convulsions and tremors, and

hazardous to human being by inhalation of solvent into the lungs (chemical pneumonitis). Besides that, lambda cyhalothrin is also harmful to fish and honey bees.

However, with the usual practise of lambda cyhalothrin by workers or people, that have a good work practices and applying safety precautions, it can be noted that lambda cyhalothrin is safe to be used, as their exposures are really low and unlikely to represent a hazard. Despite reported toxicity to fish, bees, aquatic anthropods in laboratory and in the real environments, lasting effects are not likely to occur under recommended conditions of use (WHO, 1990).

2.4.6.1 Inhalation Exposure

2.4.6.1.1 Death

Two cases involving human exposure to pyrethrin which result in deaths, were reported before, involving an allergic reaction towards a dog syampoo products containing pyrethrins (Wax & Hoffman, 1994). First cases involved an 11 year old girl which was diagnosed with asthma at 6 years old. Less than 10 minutes, subject suffered a severe acute asthmatic attack and died in less than 3 hours later, after she started to wash her dog with a shampoo containing 0.2% pyrethrin, even with medical treatment given to the girl (Wagner, 2000). Almost the same case happened to a 37 year old female, which developed severe shortness of breath, 5 minutes after beginning to wash her dog with a shampoo containing 0.06% pyrethrin (Wax and Hoffman, 1994). Despite efforts in saving her, subject died a short time later. Further postmortem examination show a pulmonary findings consistent with reactive airway responses. Beside this two cases, there are no other reports involving death due to inhalation exposure to pyrethrins or pyrethroids (ATSDR, 2003).

Inhalation exposure studies towards animals were done before and reported that most of synthetic pyrethroids are more toxic than natural pyrethrins (the active neurotoxic components of pyrethrum extract). Based on studies on rats exposure to

synthetic pyrethroids indicate that LC50 values ranging from approximately 23 to 1,000 mg/m^3 (Curry & Bennett, 1985; Flucke & Thyssen, 1980; Hext, 1987; Kavlock *et al.,* 1979; Pauluhn & Thyssen, 1982). Another study focused on the lethality of several type I pyrethroids (Miyamoto, 1976). This study produced negative results in term of lethality following exposure to airborne pyrethroid concentrations ranging from 685 to 2,500 mg/m^3.

There was only one case regarding death towards mice and rats that were exposed to a mixture of (+)-allethronyl (+)-trans allethrin for 3 hours. In another study, Miyamoto (1976) studied on the toxicity of several Type I pyrethroids in rats and mice. Both subjects were exposed to pyrethroid atmospheres in a concentration ranging from 6.1 to 210 mg/m^3, for a period of time (2–4 hours/day, 5 days/week for 4 weeks). Result from this study indicated clinical signs of toxicity at concentrations of 61.3 mg/m^3 (allethrin) and 200 mg/m^3 (furamethrin), but no death cases were reported during the study period **(**ATSDR, 2003).

2.4.6.1.2 Respiratory Effects

Some respiratory effects are reported from human use of pyrethroids based products. Eleven complaint on nasal irritation and six complaint of throat irritation based on indoors spraying of lambda cyhalothrin for successive 6 days were reported amongst twelve workers (Moretto, 1991). Pyrethroid use on conifer seedlings had some effects to the workers, who reported sign of respiratory effects by coughing, dyspnea, increased nasal secretions and sneezing in plant nursery (Kolmodin-Hedman *et al.,* 1982). Sniffles and sneezes were noted in subjects exposed to deltamethrin and fenvalerate while packaging the insecticides (He *et al.,* 1988).

2.4.6.2 Oral Exposure

2.4.6.2.1 Death

Direct oral exposure towards pyrethroid involves serious cases with high number of reports involving death cases. A case happened in China between 1983 and 1988 with 573 cases involving acute pyrethroid poisoning. From those 573 cases, 344 cases were due to ingestion of pyrethroid with four cases of death, with two of it were related to occupational exposure (He *et al.,* 1989). In 1996, Peter *et al.* (1996) reported a death of a 30 year old male after direct consumption of about 30 ml deltamethrin in two days. Unintention ingestion of unknown containing 10% cypermethrin, caused an adult male to rapidly develop convulsions, became comatose, and died shortly after that (Poulos *et al.,* 1982).

Laboratory studies on animals reported a number of death cases involving animals. One of six dogs who was given a daily oral doses of cyhalothrin at 3.5 mg/kg, was killed due to persistent pyrethroid-induced convulsions after 46 weeks in 52 weeks of oral dosing study (Hext *et al.*, 1986). Loss of life also reported to rats that were being treated with permethrin in their diets during a 90 day oral exposure (DOD, 1977).

2.4.6.2.2 Gastrointestinal Effects.

For human subjects, gastrointestinal effects due to oral exposure cases were only limited to clinical signs after some exposure to pyrethroids. Symptoms only appeared after consumption of relatively large quantities of pyrethroids with epigastric pain, nausea, vomiting and diarrhea appeared indicating the effects of pyrethroid exposure towards human (Gotoh *et al.,* 1998; He *et al.,* 1989).

In animals studies, a diet containing pyrethroids at a dose level of 1-6 mg/kg/day shows a present of diarrhea in dogs for treatment periods ranging from 13 weeks to one year (EPA, 1981; IRIS, 2003a, 2003b, 2003c).While, the presence of esfenvalerate in

male mice diets showed gastritis and mucosal erosion and ulceration in study for 90 days at mean concentration of 106 mg/kg/day (EPA, 1991).

2.4.6.2.3 Immunological and Lymphoreticular Effects

Studies on immunological and lymphoreticular effects of pyrethroid involved parts of body that were related to the immune system, lympoid and reticuloendothelial systems. Studies are carried out in rats and rabbits for twelve weeks with cypermethrin at 6.25, 12.5, or 25 mg/kg/day. The results of this study show some changes to the subjects with decreases in rosette-forming lymphocytes, relative spleen weight and ratio of lymphocytes to leukocytes with suppression of the humoral immune response (Desi *et al.*, 1986).

Another study was done with addition of 40 mg/kg/day cypermethrin orally in male rats that revealed a severe leukopenia in a 90 days study (Varshneya *et al.,* 1992). Madsen *et al.* (1996) observed the increasing number of antibody forming cells in the spleen and enhanced natural killer cell activity after oral administration to the rats at 5 or 10 mg/kg/day for 28 days.

2.4.6.2.4 Neurological Effects

Some studies were done to look on the neurological effects of laboratory animals towards administration of pyrethroids orally. For neurological effects, there are different depending on the types of pyrethroid shown by the symptoms appeared to the animals. For type I pyrethroids, some of the behaviour shown to have relationship with neurological effects including aggressive behavior and increased sensitivity to external stimuli, fine tremor, prostration with coarse whole body tremor, elevated body temperature and coma (Mbaria *et al.,* 1993; Schoenig 1995).

Differents in symptoms shown by type II pyrethroids are mainly due to the chemical structures of the pyrethroid itself. Most of type II pyrethroids have a cyano group which are absent in type I, thus, showing different symptoms like pawing and

burrowing behaviour, profuse salivation, increased startle response, abnormal hindlimb movements, and causes whole body to tremor that progresses to sinuous writhing (choreoathetosis) (Miyamoto *et al.,* 1995; Wright *et al.,* 1988). Most of the studies with Type I or Type II pyrethroids by oral dosing have shown similar trends with clinical signs of neurotoxicity within minutes to hours and symptoms shown for several hours to a few days (EPA, 1992; Eriksson & Nordberg, 1990; Hudson *et al.,* 1986; Parker *et al.,* 1983, 1984a, 1984b; Ray & Cremer, 1979; Southwood, 1984).

Some of the researchers tried to look on other aspects of neurotoxicity in animals, who were administered with oral doses of pyrethroids. But, most of their findings resulting in much lower concentration than typical clinical sign. For example, studies on the motor activity of rats following administration of Type I pyrethroid (permethrin) at 200 mg/kg and Type II pyrethroids (cyfluthrin at 12.5 mg/kg, fenvalerate at 30 mg/kg, flucythrinate at 2.5 mg/kg, cypermethrin at 30 mg/kg and fluvalinate at 15 mg/kg, showing a significant decrease in their motor activity (Crofton & Reiter, 1988).

2.4.7 Degradation of Lambda Cyhalothrin

Pyrethoid degrades through several process in natural environment. The most common pathways are:

i) photodegradation
ii) biodegradation
iii) hydrolysis

Amongst these pathways, photodegradation is known as the most common degradation pathway with faster and higher degradation rate as compared to biodegradation and hydrolysis (Liu *et al.,* 2010).

2.4.7.1 Photolysis

The photodegradation process of lambda cyhalothrin was studied in exposure towards UV light. In this study, Ruzo (1982) concluded that photodegradation of lambda cyhalothrin followed pseudo first order kinetic under direct irradation. Lambda cyhalothrin degradation shows the degradation products of a, b, c, d, phenoxyphenylacetonitrile (h) and 4-phenoxybenzoic acid methyl ester (i) (Figure 2.2). Based on figure 2.2, chemical mechanism involved in photodegradation of lambda cyhalothrin involved ester bond cleavage, C–C cleavage, photooxidation and photoisomerization. The structure of lambda cyhalothrin that contain isobutyl, double bond and other unstable groups enable cleavage to occur at these points via photooxidation (Ruzo,1982).

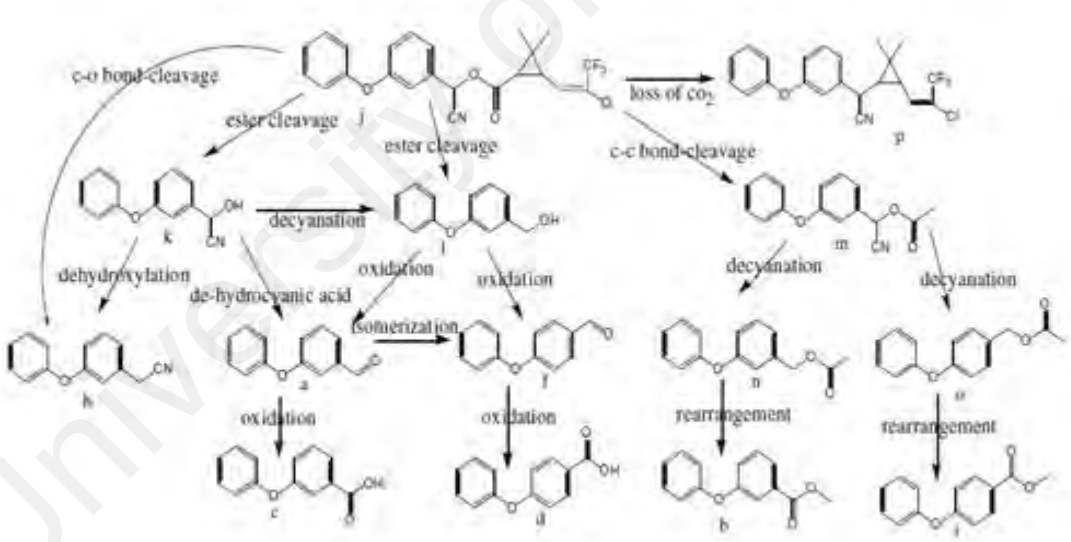

Figure 2.2: Suggested degradation pathways of lamba cyhalothrin through photodegradation (Liu *et al.,* 2014)

Process in obtaining photoproduct (a) was done by dehyanic acid of 3-phenoxyphenyl acetonitrile (k) and oxidation of 3-phenoxybenzyl alcohol (l), while photoproduct (c) and (d) can be obtained from photooxidation of (a) and (f),

respectively. Molecular rearrangement and photoisomerization of (m) will earn photoproducts (b) and (i). Besides that, photoproduct (h) was formed from ester bond cleavage and then dehydroxylation or directly by C–O bond cleavage due to electron attraction of fluorine and chlorine in the photodegradation process.

Photoproducts (b) and (i) were present in small quantities, so the C–C bond cleavage and rearrangement route were not major parts of the mechanism. Small amounts of (h) were also detected in the above solution. Photoproduct (h) was generated directly by C–O bond cleavage or by ester bond cleavage and then dehydroxylation. Therefore, dehydroxylation and C–O bond cleavage did not play a key role in the photodegradation of the pyrethroid. Therefore, it is concluded that photooxidation was the major process for photodegradation of lambda cyhalothrin in the present of UV light (Liu *et al.,* 2014).

2.4.7.2 Hydrolysis

Lambda cyhalothrin has a possibility to undergo hydrolysis due to the stability of lambda cyhalothrin at pH below 8 that are under alkaline solution. Hydrolysis of lambda cyhalothrin will occur through nucleophilic attack of the hydroxyl ion forming a derivative of cyanohydrin. This derivative will degrade to form hydrogen cyanide (HCN) and corresponding aldehyde (Figure 2.8) (Gupta *et al.,* 1998).

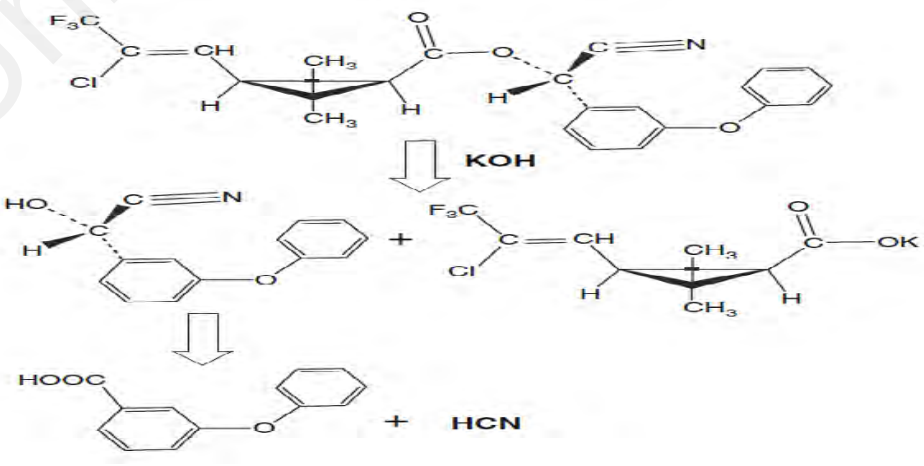

Figure 2.8: Products of lambda cyhalothrin hydrolysis (He *et al.,* 2008)

2.4.7.3 Microbial Degradation

Study done comparing a non sterile soil to sterile soil indicate biodegradation process happening as lambda cyhalothrin degrade rapidly in non sterile soil (Wang *et al.,* 1997). The major degradation product of lambda cyhalothrin was (*R,S*)-α-cyano-3-(4 hydroxyphenoxy)benzyl-(*Z*)-(1*RS*)-*cis*-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2dimethyl -cyclopropanecarboxylate, that are 10% from the initial lambda-cyhalothrin concentration (European-Commission, 2001).

CHAPTER 3: VERIFICATION AND VALIDATION OF METHOD DEVELOPMENT FOR DETERMINATION OF LAMBDA CYHALOTHRIN IN OIL PALM PLANTATION (CRUDE PALM OIL, CRUDE PALM KERNEL OIL, LEAVES, WATER AND SOIL)

3.1 Introduction

Method development is one of key interest amongst researchers, as this type of research will keep growing due to continuity in looking on the simplest, quality determination or detection in improving research in the field of analytical chemistry and environmental studies. As research continues, there are several organisations and goverment bodies that monitor and develop a standard procedure to be used by analyst. This standard procedure or any new method development usually will proceed with a method validation, as the method needs to demonstrate 'fitness to purpose', whereby this method will be used and produce valuable data for other analysts. Method validation can be comprehended based on several definitions. Eurachem defines method validation as 'the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires' (Eurachem, 2014). While, ISO describes validation as 'confirmation through examination and provision of objective evidence that the requirements for a specified intended use or application are fulfilled' (International Organization for Standardization, 2005).

Method validation is categorized based on the purpose of the works. In-house validation is usually done on newly developed methods (Thompson *et al.,* 2002). In-house validation is performed after method development and will become a routine analysis in the laboratories as long as the method is used. This will ensure a continuous good performance by the method in the future. Another types of validation is known as inter-laboratory validation. This type of validation has two purposes which are, to obtain the typical performance characteristics and systematic errors of the method. Both

are done in a single method with collaboration between different laboratories (AOAC International, 1995). To simplify, method validation is expressly conducted for testing the performance of new method development, revising established method or method done in different labarotories, analyst or instrumentation (Eurachem Guide, 1998).

Researchers, industries and authorities benefits from the present method validation guidelines from variety of worldwide organisations and agencies. Among them are Eurachem (Eurachem Guide, 1998), the International Union of Pure and Applied Chemistry (IUPAC), AOAC (AOAC International, 1995), the U.S. Food and Drug Administration (FDA), The United States Pharmacopoeia (USP; *United States Pharmacopoeia*), International Conference on Harmonization (ICH, 2005) and World Health Organization (WHO). These agency guidelines serve as an indicator for a good and well developed method to be used by researchers (Bonfilio *et al.,* 2012). An important aspect of method validation is to ensure that any analytical method is correctly used and provide a reliable results (Thompson *et al.,* 2002; Hubert *et al.,* 2003; Ribani *et al.,* 2004).

Confirmation of lambda cyhalothrin is done using gas chromatography with mass spectrometry detector (GC-MS). Gas chromatography (GC) is an important and significant instrument in analytical chemistry world from late 1950s and early 1960s. Eventhough it was still new at that time, but the introduction of GC-MS was done during this period of time (Gohlke *et al.,* 1993; Holmes *et al.,* 1957). Mass spectrometry had long been used as an important tool in identification and detection of molecule**.** The first MS applications involved discovering of isotopes and counting their relatives abundance. In 1935, MS managed to analyze all elements from periodic table based on their isotopic composition (Grayson *et al.,* 2002). From there, MS have grown in

becoming the big player, as the world are driven from intensive petroleum and pharmaceutical industries, with characterization of refined petrochemicals and natural products. Gas chromatography is known for its ability to efficiently separate a complex mixtures, but lack in identifying each one of them. This shortfall was removed by the present of MS with its strength in identification of molecule based on their fragmentation pattern. The combination of the two techniques were in a way complementing each other, while utilizing their benefits and lessen the limitations in each of them. Nowdays, GC-MS and GC-MS/MS are used for the environmental and food analyses studies. With high resolution MS techniques present, such as time-of-flight MS (TOF-MS), the identification studies are more detailed in dealing with a complex structure confirmation. In this study, gas chromatography-mass spectrometry (GC-MS) is used in identification of lambda cyhalothrin in palm oil agrosystem samples.

Therefore, method validation serves as an important parameter when using newly method or revising an established method in providing the basic requirement of a good and reliable analysis with those methods involved. In this study, five parameters for validation were done on establised method for detection of lambda cyhalothrin in palm oil plantation including method of detection in crude palm oil and palm kernel oil, soil, leaf and water samples. These parameters include identification and confirmation for analyte of interest, linearity, selectivity, recovery and detection limit of instrument.

3.2 Experimental

3.2.1 Reagents and Materials

The analytical grade of lambda cyhalothrin (purity 99 %) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). All the solvents of HPLC grade and chemicals of analytical grade were purchased from Merck (Darmstadt, Germany). Reagent grade anhydrous $MgSO_4$ was obtained from Supelco Inc. (Bellefonte, PA, USA). Extracts were filtered using filter paper and syringe filter (nylon, 0.45 µm) and both were purchased from Whatman (Maidstone, Kent, UK).

SPE cartridges used for the cleanup step were graphitized carbon black (Carbograph, 500 mg/6 ml) obtained from Alltech Inc. (Deerfield, IL, USA). C18 (LiChrolut RP-18, 500 mg/6 ml) was purchased from Merck KgaA (Darmstadt, Germany), while primary secondary amine (PSA, 500 mg/2 ml) was supplied by International Sorbent Technology (Hengoed, Mid-Glamorgan, UK).

3.2.2 Apparatus and Glassware

A vortex mixer used in the sample extraction and partition step, was obtained from Barnstead/Thermolyne Inc. (Dubuque, IA, USA). Ultrasonicator used in samples extraction step was obtained from Branson 5510 (Danbury, CT, USA) and N-Evap nitrogen evaporator for sample concentration was acquired from Organomation Associates Inc. (South Berlin, MA, USA). Mircoliter pipettes, adjustable between 100 and 1000 µL, and pipette tips were obtained from Eppendorf (Hamburg,Germany), while SPE vacuum manifold was from Supelco Inc. (Bellefonte, PA, USA). Mircovials (2 ml) for GC injection were purchased from Agilent (Palo Alto, CA, USA). Ten mL graduated vials that used to collect the analytes eluted from the SPE cartridges were obtained from Alltech Inc. (Deerfield, IL, USA). N-Evap nitrogen evaporator for sample concentration was from Organomation Associates Inc. (South Berlin, MA, USA).

3.2.3 Instrumentation

3.2.3.1 Gas Chromatography with Micro Electron Capture Detector (GC-µECD)

Sample extracts were analyzed using Agilent Model 6890 series gas chromatograph equipped with a 7683 auto-sampler, split/splitless injector and an µECD operated at 280°C (Agilent Technologies). The injection mode was splitless, whereby it operated at 250ºC, and the injection volume was 2.0 µl. A DB-608 column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Agilent Technologies) was used to separate the analytes. Nitrogen was used as the carrier and makeup gas, with the flow rate for carrier gas and makeup gas at 1.2 ml/min and 60 ml/min respectively. The initial temperature was 150ºC, with the initial time of 2 min. The oven was heated to 250ºC at 20ºC/min and then held for 25 min**.** Chemstation software was used for instrument control and data analysis.

3.3.3.2 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry was used to confirm lambda cyhalothrin chromatogram obtained from GC-µECD. GC-MS used was Agilent 7890A coupled with an Agilent 5975C mass spectrometer. Data handling and system operations were controlled by GC-MS NIST05 and RTLPEST3.L software. Separation was carried out using an HP-5MS column (30 m length x 0.32 mm i.d. x 0.25 µm). For the chromatographic determination, purified helium (99.99%) was used as carrier gas at a constant flow rate of 1.2 ml/min. Injector temperature was kept at 250ºC with splitless mode and injection volume of 5.0 µl. The oven temperature programming was started with initial temperature at 100ºC (held for 1 min), ramped at 10 ºC/min to 250ºC and then to 3ºC/min to 280ºC (held for 15 min). The full scan of electron ionization mass spectra was obtained under conditions of mass-to-charge ratio (m/z) scan range from 30.0 to 500.0, and solvent delay was 3 min. The GC-MS interface and ion source temperature were set at 150ºC and 230ºC, respectively.

3.3.4 Preparation of Stock Standard Solutions

Individual stock standard solutions of lambda cyhalothrin was prepared in acetone at concentration of 2000 µg/g by dissolving 0.1 g of lambda cyhalothrin in 50 ml acetone and refrigerated at -20ºC in amber glass-stopped bottles in the dark. Then, intermediate working standard solutions were prepared by dilution of the stock solutions in acetone to give pesticide standards of 100 µg/g and 10 µg/g. Finally, serial dilutions of the working standard solutions were performed to give seven calibration solutions (0.01, 0.02, 0.05, 0.08, 0.1, 0.5, 1 µg/g) in acetone. All the standard solutions were stored in scintillation vials at 4ºC in the refrigerator.

3.3.5 Method for Determination of Lambda cyahlothrin in Oil Palm Plantation

3.3.5.1 Analytical Procedure for Crude Palm Oil and Palm Kernel Oil

Samples were analyzed according to Halimah *et al.* (2012). Samples of 5.0 g homogenous oil (CPO or CPKO) were transferred into 50-ml screw cap test tubes. Samples were mixed well using a vortex mixer and allowed to stand for 30 min for equilibration. Then, 10 ml of acetonitrile was added into the fortified samples in each tube and mixtures were shaken for 5 min using a vortex mixer. The oil precipitated at the bottom of the test tubes, and the acetonitrile extract rose to the top. Each tube was kept horizontally in a freezer (-20°C) for 2 hour for oil precipitation before undergoing clean-up procedure. SPE cartridges were first conditioned with 5 ml of acetonitrile. An aliquot (2 ml) of the upper layer of the acetonitrile extract from the low-temperature precipitation step was transferred into the cartridge. The extract was initially allowed to flow under gravity. Then a gentle pressure was applied to achieve a flow of approximately one drop per second. The eluate was collected at this point into a 10-ml graduated vial. The column was then eluted with an additional acetonitrile and the volume of eluate collected was adjusted to 5 ml. Finally, the eluate was homogenised and ready for GC analysis (Halimah *et al.,* 2012a).

3.3.5.2 Analytical Procedure for Leaves

Five gram of leaves sample was weighed in 100 ml beaker. Then, 100 ml of acetonitrile was added and sample was extracted by ultrasonic extraction using ultrasonic bath for 30 min. 5 g of sodium chloride was added to the flask to increase the extraction efficiency. After the extraction period, the extract was decanted and filtered through a piece of Whatman filter paper (filled with approximately 1 g of anhydrous $MgSO_4$) into a 20 ml scintillation vial. An aliquot (20% of the original volume) was transferred into scintillation vial and evaporated to dryness using N-evaporator. Next, 2 ml of acetonitrile was added to the vials and loaded into pre-conditioned GCB cartridge and concurrently an adaptor is attached into the top of the pre-conditioned PSA catridge reservoir. The extract was allowed to flow and a gentle pressure is applied to achieve a flow of approximately one drop per second. Collection of the eluate began at this point into graduated vial. The column was the eluted with 20 ml acetonitile : toluene (3:1). Eluate was then evaporated to dryness using nitrogen. Finally, the extract was reconstituted with 1 ml of acetone prior to the GC analysis.

3.3.5.3 Analytical Procedure for Soil

Homogenized soil (20 g) was weighed in 100 ml beaker. Then, 25 ml of acetonitrile was added and the sample was extracted by ultrasonic extraction. After the extraction period, the extract was decanted and filtered through a piece of Whatman filter paper (filled with approximately 1 g of anhydrous $MgSO_4$) into a 20 ml scintillation vial. Then, the extract was again filtered through a nylon syringe filter to remove additional fine soil particles to obtain a clear solution. An aliquot (20% of the original volume) was transferred into scintillation vial and evaporated to dryness using N-evaporator. The extract was then finally reconstituted with 1 ml of acetone and ready for GC analysis.

3.3.5.4 Analytical Procedure for Water

One litre of water samples (1 L) was allowed to flow into pre conditioned (3 ml acetonitrile + 3 ml water) C_{18} cartridge until it finished up. The column is eluted with 8 ml methanol. Eluate was then evaporated to dryness using nitrogen evaporator.The extract was then finally reconstituted with 1 ml of acetone and ready for GC analysis.

3.4 Results and Discussion

3.4.1 Identification of Lambda Cyhalothrin using Gas Chromatography with Micro Electron Capture Detector (GC-μECD) and Gas Chromatography-Mass Spectrometry (GC-MS)

Confirmation of lambda cyhalothrin is one of the important aspects in monitoring studies especially dealing with the real samples in environment. The GC-MS product ion of lambda cyhalothrin for the spiked sample and the mass spectrum of detected lambda cyhalothrin from MS library are shown in Table 3.1 and Figure 3.1 respectively. The mass spectrum of lambda cyhalothrin detected in the pure standard acetone was confirmed by matching with mass spectrum from the MS library. Based on the MS library and GC-MS product ion of several authors (Amrazi *et al.,* 2009; Melo *et al.,* 2012) the product ion of lambda cyhalothrin was as follows:

Table 3.1: Product ion of lambda cyhalothrin standard
(Amrazi *et al.,* 2009; Melo *et al.,* 2012; Stefanelli *et al.,* 2009)

Compund	Quantifying Ion	Qualifying Ion 1	Qualifying Ion 2
Lambda cyhalothrin	181 (100)	197 (65)	208 (28)

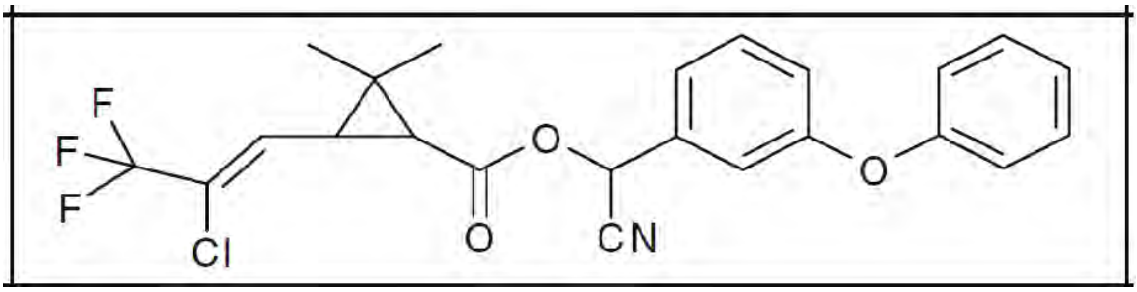

Figure 3.1: Structure of lambda cyhalothrin (Casas *et al.,* 2007)

Using gas chromatography-mass spectrometry (GC-MS), lambda cyhalotrin was identified and compared with product ion of lambda cyhalothrin from Table 3.1. Based on Figure 3.2, it is shown that, the product ion of lambda cyhalothrin is similar to the reading in Table 3.1. Therefore, it can be concluded that lambda cyhalothrin is identified for this study.

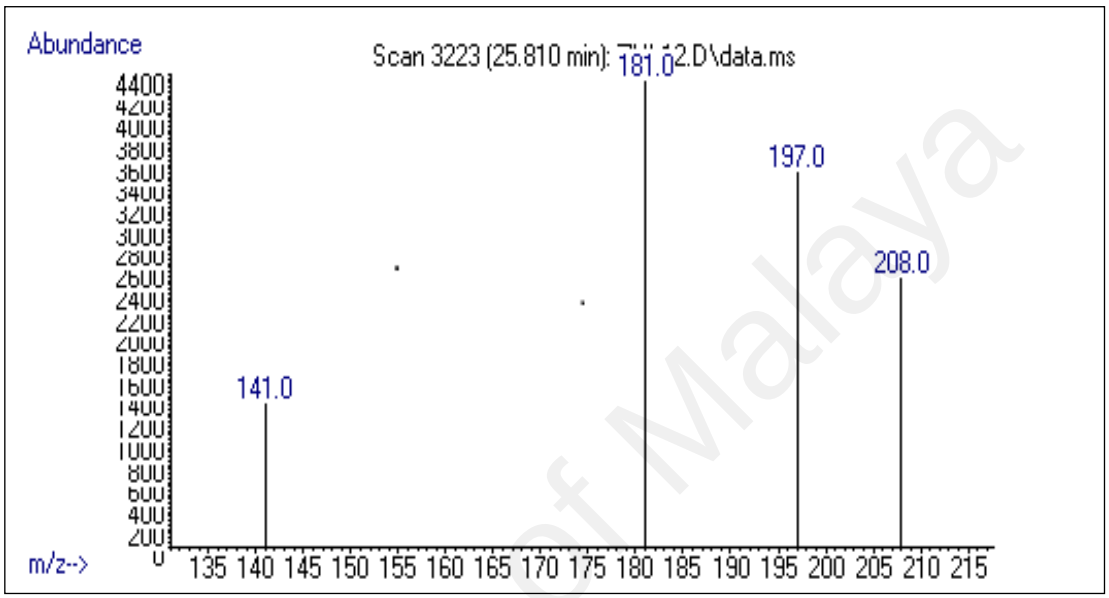

Figure 3.2: Mass spectra of lambda cyhalothrin obtained from experiment.

The identity of lambda cyhalothrin was confirmed in the pure standard acetone. This identification and confirmation of residues are crucial as it is generally used for human health and environment protection. This also follows the EU criteria as 'Confirmatory methods' for analytical procedures to be accepted (Careri & Mangia, 2006).

3.4.2 Verification and validation method for determination of Lambda Cyhalothrin in Oil Palm Plantation (Crude Palm Oil, Crude Palm Kernel Oil, Fronds, Water and Soil)

3.4.2.1 Linearity

Linearity is an aspect of method validation. Linearity can be defined as 'the ability of an analytical procedure to obtain results that are directly proportional to the analyte concentration in the sample within a given range (ICH, 2005). According to Australian

Pesticide & Veterinary Medicine Authority (APVMA, 2004), linearity is the ability of analytical procedure to produce test results which are proportional to the concentration (amount) of analyte in the samples within a given concentration range, either directly or by means of a well-defined mathematical transformation. In linearity test, there will be existence of the working range with a linear relationship between analyte concentration and signal response.

Linearity is usually done by establishing calibration curves, either by the presence or absence of sample matrix by diluting the standard analyte in different levels of concentration covering the whole working range. The suggested working range differs according to different organisations. Five different levels with expected concentration within the ranges of 80 to 120% were suggested by the USP (United States Pharmacopoeia) and ICH (2005). Meanwhile, AOAC (2000) recommends four different levels along three different days (target concentrations of 50, 100, 150 and 200% of the test concentration) for linearity test. IUPAC recommends six different concentrations level within concentration range of 0 to 150% or 50 to 150% and APVMA suggests using a minimum of six standards with concentration within 80 –120% of the expected concentration range (APVMA, 2004; Bonfilio *et al.,* 2012).

The calibration curve was constructed using seven points (IUPAC & APVMA) based on the area measurement in the working range of (0.01- 1 µg/g) for lambda cyhalothrin concentration (Figure 3.3). The linearity of method was evaluated by linear regression analysis calculated by least square method. The linear equation for lambda cyhalothrin standard was y = 272861x-3552.3 with y is the area of the lambda cyhalothrin peak obtained from the GC-µECD analysis and *x* is the concentration of lambda cyhalothrin in µg/g. The coefficient of correlation (R^2) for the calibration curves was 0.994, indicating the linearity of the calibration curve plotted for this study (Figure 3.3).

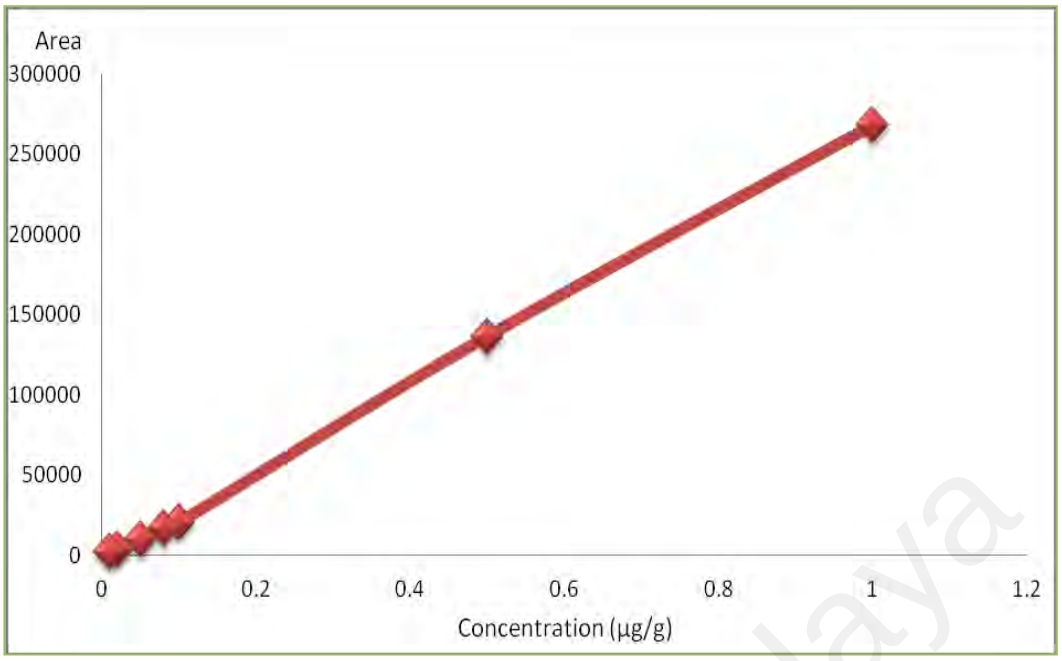

Figure 3.3: Calibration curves for lambda cyhalothrin (0.01- 1 µg/g) n=7

3.4.2.2 Accuracy

Accuracy or recovery of a method is the closeness of the analyte in a sample to its true value (Green, 1996; APVMA, 2004; Bonfilio *et al.,* 2012). While precision can be defined as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (APVMA, 2004). In determining the accuracy of method, several guidelines provided to assist researchers in their works. There are three common ways of producing a recovery study. One is by direct comparing of known sample concentration to its true standard value. However, the standard used must be well characterised reference standard (ICH, 2005).

The second method is known as spike-placebo (product matrix) recovery method. This method emphasis on the spiking of known amount of reference standard to a matrix. Then, a comparison is made with the expected result of known reference standard. Standard addition method are the third method that can be choosen by the researchers. In this method, sample or matrix is analysed first before the addition of reference standard known as unspiked sample. Sample is analysed again, but now with a

standard reference added into it (spiked sample). Then, the difference between the two spiked and unspiked samples are compared with the expected result. Recovery is based on the ratio of the observed result to the expected result expressed as a percentage in standard addition and spike-placebo (product matrix) method (APVMA, 2004). ICH (2005) suggests nine measurements for at least three different concentrations (low, medium and high concentration) for accuracy determination. In this study, FDA (U.S. Food and Drug Administration, 2000) method was followed with accuracy studies done on three concentration levels for five replicates for each level (Low, medium and high) (APVMA, 2004; Bonfilio *et al.,* 2012). The mean recoveries of lambda cyhalothrin were found to be 87-90%, 96-109%, 81-105% and 83-90% respectively, for crude palm and palm kernel oil, leaves, soil and water sample at three level of concentrations (Table 3.2). The relative standard deviation (RSD) between 8.6-15%, 1.6-10.9%, 5.80-6.2% and 4.2-7.6% for all samples demonstrates the good performance of the methods (Table 3.2).

Table 3.2: Recovery (%) and relative standard deviation (RSD) (%) of lambda cyhalothrin in oil palm agroenvironment samples

Sample	Fortified level (mg/kg)	Mean Recovery (%)	RSD (%)
Crude palm & palm kernel oil	0.02	90.0	8.6
	0.05	87	15
	1	90	9.8
Frond / leaves	0.02	102	10.9
	0.05	109	4.4
	1	96	1.6
Soil	0.02	81	5.8
	0.05	95	5.9
	1	105	6.2
Water	0.02	83	4.2
	0.05	86	5.3
	1	90	7.6

3.4.2.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

When an analysis is done in determining the analyte of interest, it is crucial to know the level of one detection that can be performed experimentally. This is important in trace analysis where such low level of ppm and ppb concentration is being analysed (Eurachem, 1998). The information on the lowest detection will guide the researcher on the ability and limit of their instrument to perform analysis on their analyte of interest. Therefore, two parameters were provided in indicating the lowest detection for analyte analysis. Those two are known as limit of detection (LOD) and limit of quantification (LOQ). Based on APVMA guideline, limit of detection can be defined as 'the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value'. As for limit of quantification, it is 'the lowest amount of the analyte in

the sample that can be quantitatively determined with defined precision under the stated experimental conditions'. The limit of quantification is a 'parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities or degradation products or low levels of active constituent in a product' (APVMA, 2004). There are several ways in determining the limit of detection and limit of quantification.The first method is based on the signal to noise ratio where the low analyte concentrations are compared to signals of background noise. Based on several low concentrations tested, the minimum concentration that can be detected are selected and establish as the limit of detection and limit of quantification. Another possibility is by producing the analyte calibration curve and looking on the slope and standard deviation of the curves to determine the limit of detection and limit of quantification (Epshtein, 2004).

In the present study, the signal to noise (S/N) ratio approach was used, where signals from lambda cyhalothrin standard were compared with blank sample. LOD was determined by analyzing the decreasing concentration of analytes spiked on sample matrix until obtaining a signal-to-noise (S/N) ratio of 3 while LOQ was derived from LOD values to give an S/N of 10. LOD for the proposed method was found to be 0.002 µg/g for lambda cyhalothrin and 0.02 µg/g for LOQ determination (Figure 3.4). LOQ is the lowest amount of analyte that can be measured with sufficient precision and accuracy of method. The S/N of 10:1 is commonly accepted for limit of quantification (Taylor *et al.,* 2006). The low detection limits indicates that gas chromatography-electron capture detector is capable of sensitive quantification of lambda cyhalothrin in the environment.

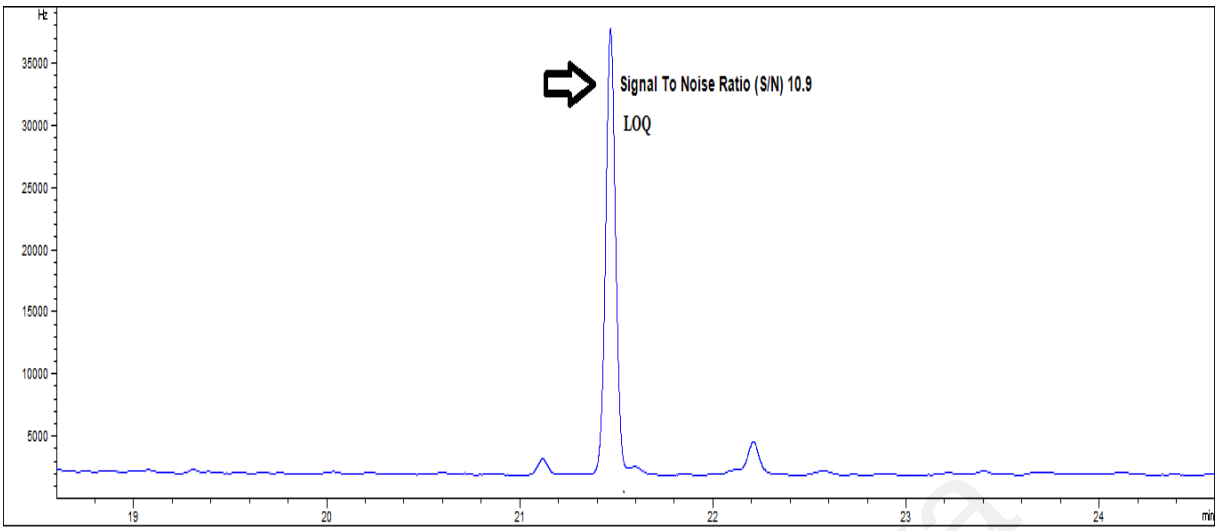

Figure 3.4: Chromatogram of limit of quantification for lambda cyhalothrin (0.02 µg/g at 21.4 min) based on signal to noise ratio.

The LOQ value of 0.02 µg/g for lambda cyhalothrin in palm oil matrices using gas chromatography with micro electron capture detector (gc-µecd) can be compared with previous studies. Study on olive oil using gas chromatography with electron capture detector (gc-ecd) over a period of 3 years time resulting in the same value of 0.02 µg/g for LOQ determination of lambda cyhalothrin in olive oil (S/N ratio method). A higher LOQ was presented in the previous study on tomato matrices using gas chromatography-mass spectrometry with 0.08 µg/g (standard deviation of response and slope method). In another study looking on dissipation kinetics of lambda cyhalothrin in cardamom, show a higher LOQ value with 0.025 µg/g for LOQ determination using gas chromatography and electron capture detector (gc-ecd) (S/N ratio method).

Beside that, there are several studies showing a lower value of LOQ compared to this study at 0.02 µg/g. First, study on determination of green tea leaves using gas chromatography with micro electron capture detector (gc-µECD) show a lower LOQ of 0.015 µg/g (S/N ratio method). Looking on the rate of lambda cyhalothrin degradation in grapes (*vitis vinifere L.*) revealed an LOQ value of 0.01 µg/g with gas chromatography-mass spectrometry

(S/N ratio method). Another two previous studies in strawberries using liquid chromatography-MS/MS and on tomato (S/N ratio method) matrices using gas chromatography and electron capture detector (gc-μECD) shows a lower LOQ values of 0.005 μg/g and 0.0125 μg/g. Signal to noise (S/N) ratio method use in this study and most of previous studies before are based on the instrumental properties. S/N method have its own advantages compared to other methods, as this method includes all errors resulting from sample matrix, method use, calibration and the instrument itself.

3.4.2.4 Selectivity

Selectivity of a method according to APVMA was an extent in which 'it identifies particular analyte(s) in a complex mixture without interference from other components in the mixture'. The terms selectivity and specificity are commonly used in reference for the same work. Some might differentiate both terms separately to its own separate meaning. Based on APVMA, 'The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other'. Method can be said to be selective if the analyte of interest able to differentiate from other responses or interferences. The International Union of Pure and Applied Chemistry (IUPAC) deduced that "Specificity is the ultimate of Selectivity'. Therefore, IUPAC suggested the use of the term selectivity instead of the term specificity (APVMA, 2004). Nevertheless, both terms are considered same for some authors (Taverniers *et al.,* 2004).

Therefore, selectivity is studied on four matrices (crude palm and palm kernel oil, leaf, soil and water) towards lambda cyhalothrin standard. Based on figure 3.5 to 3.8, the chromatograms of the blank sample and matrix with analyte of interest in each matrix were shown. No inteferences disturbing the chromatogram, indicating the signal of lambda cyhalothrin was obtained at it retention time. This trends continue with the

low concentration lambda cyhalothrin producing a good separation of analyte with the background noise. This show that the method of extraction and clean up procedure in the sample of crude palm oil and leaf are selective method and the analyte of interest is well defined and separated from the interferences of sample matrix.

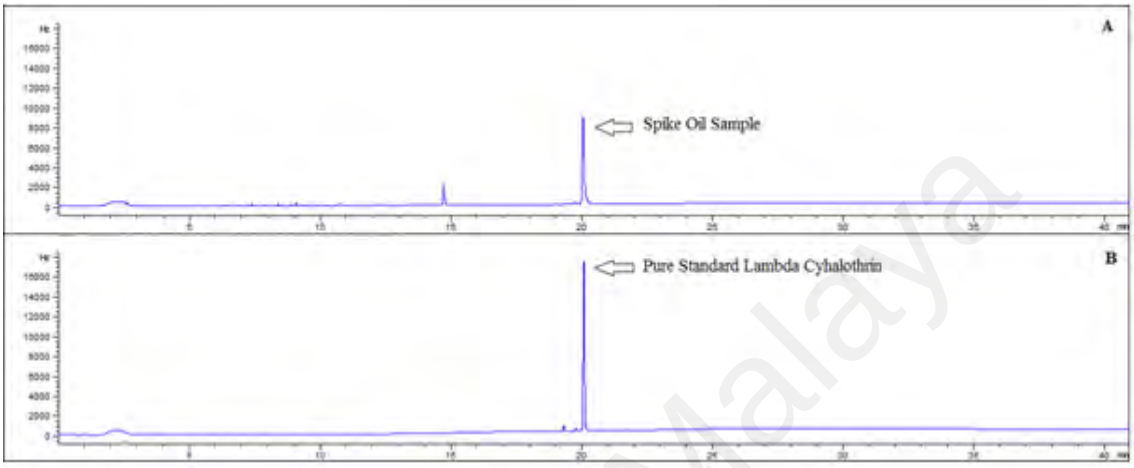

Figure 3.5**:** Chromatograms of (a) spiked oil sample (0.6 µg/g) (b) pure standard lambda cyhalothrin (1 µg/g) using gas chromatography-electron capture detector (GC-µ-ECD)

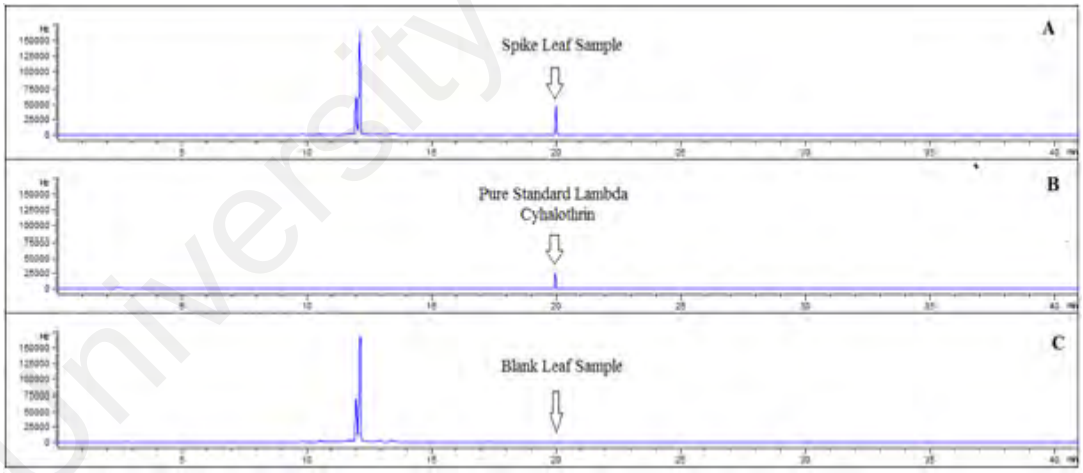

Figure 3.6: Chromatograms of (a) spiked leaves sample (0.4 µg/g) (b) pure standard lambda cyhalothrin (0.4 µg/g) (c) blank leaves sample using gas chromatography-electron capture detector (GC-µECD)

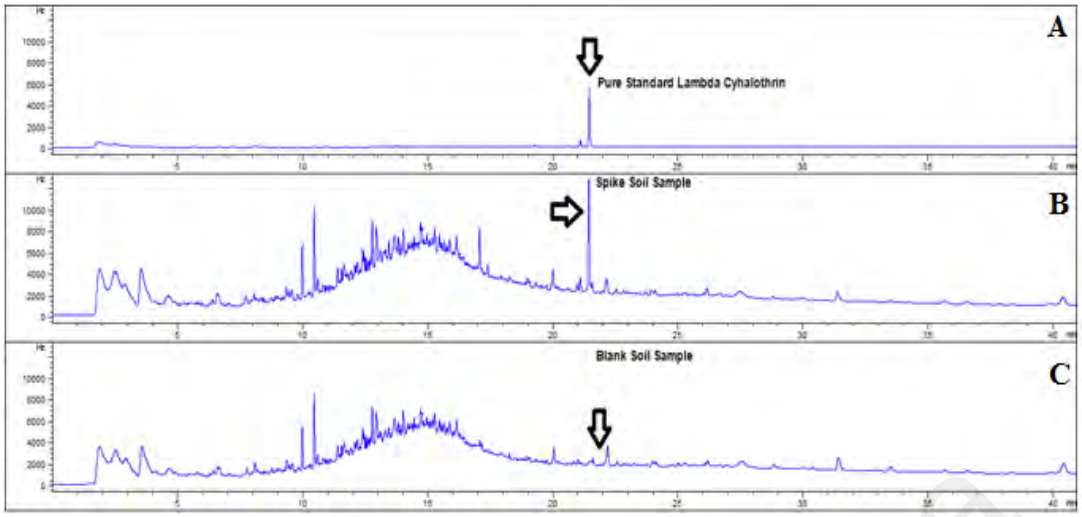

Figure 3.7: Chromatograms of (a) pure standard lambda cyhalothrin (0.4 µg/g) (b) spike soil sample (0.4 µg/g) (c) blank soil sample using gas chromatography-electron capture detector (GC-µECD)

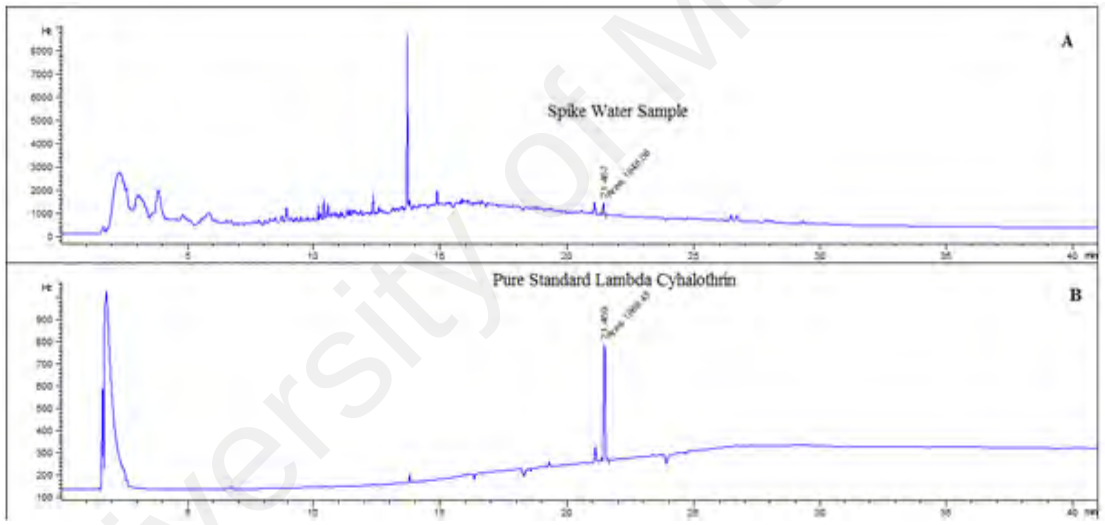

Figure 3.8: Chromatograms of (a) spike water sample (0.06 µg/g) (b) pure standard lambda cyhalothrin (0.04 µg/g) using gas chromatography-electron capture detector (GC-µECD)

3.5 Conclusion

Verification and validation of the method purposely done in ensuring the method should be able to provide a good and reliable data for researchers. For this study, five verification tests were done on the four method developed for detection of lambda cyhalothrin in Malaysian palm oil agroenvironment. The first test is identification and confirmation of lambda cyhalothrin in sample. Lambda cyhalothrin was successfully identified by using gas chromatography-mass spectrometry (GC-MS), by comparing the

product ion chromatogram of lambda cyhalothrin standard with MS library. The linearity test was able to show that method developed are capable of delivering a good test results that match the amount of analyte in different concentrations. In this study, 7 different concentrations of lambda cyhalothrin were able to provide a good linearity with coefficient of correlation (R^2) for the calibration curves was 0.994, indicating that the method developed are quantitative and adequate for lambda cyhalothrin analysis.

Selectivity is done by looking on the ability to recognize lambda cyhalothrin in the complex mixtures of other components. This really depends on the effectiveness of the method develop especially regarding the extraction and clean up methods. Based on the chromatogram obtained from GC-μECD, it shows the capability of lambda cyhalothrin standard peak to be separated from others, indicating that the method developed is a selective method. Limit of detection (LOD) and limit of quantification (LOQ) are determined based on signal to noise ratio (S/N) that are functioning as limit for one detection. LOD is 0.002 μg/g and LOQ is 0.02 μg/g for this study.

Lastly, is the recovery study that is done to know the accuracy of method developed. This study are following the FDA methods where the mean recovery of lambda cyhalothrin were found to be 87-90%, 96-109%, 81-105% and 83-90% respectively, for crude palm and palm kernel oil, leaves, soil and water sample at three level of concentrations. The relative standard deviation (RSD) between 8.6-15%, 1.6-10.9%, 5.8-6.2% and 4.2-7.6% for all samples show the good performances of the methods.

CHAPTER 4: FATE OF LAMBDA CYHALOTHRIN UNDER FIELD CONDITIONS IN OIL PALM PLANTATION

4.1 Introduction

Lambda cyhalothrin is one of the insecticide used in oil palm plantation and it belongs to pyrethroid group of chemicals. The present study were carried out to study the dissipation patterns of lambda cyhalothrin in oil palm agroenvironments and to investigate the half life of lambda cyhalothrin under oil palm field conditions.

The plantation sectors still dependent on pesticides for minimizing production loss. Cai (2008), indicated the loss of fruits, vegetables, and cereals from pests to reach 78%, 54%, and 32%, respectively, without the application of pesticide. With proper use of pesticides, the losses of crops decreased to 35–42% (Pimentel, 1997; Liu & Liu, 1999). The type and amount of pesticide used in the plantation rely on several factors including the type of pest, crop, knowledge on pest management practices and plantation economic and policy factors (Donald, 2001).

Since the use of pesticide is still relevant, several measures were undertaken by policymakers to minimize the harmful effect of pesticide including initiatives provided to the plantation sectors, policy changes and indepth study on effect of pesticide used on human health (Thomas, 2003). Focusing on public health and environmental impact assessment, policymakers addresses some of the issues that might arise from these two aspects. Some of these impacts including, exposure of pesticide to workers and public prior to pesticide spraying, exposure of pesticide in food, human health related aspects, contamination of ground and surface water for drinking and environmental impact towards life sustainability of flora and fauna (Parris & Yokoi, 2003).

Among the objectives of environmental impact assessment in environmental monitoring studies is to observe the pathway of pesticide in the environment, thus, finding a mechanism to minimize the adverse impacts of pesticide to flora, fauna and the risk exposure to human for the conservation of earth. Various reasons influenced the movement of pesticide in the environment such as topography, climatic conditions, temperature, drainage, type of crop and application method (Parris & Yokoi, 2003).

Several possible pathway for pesticide and its degradation products in the environment include atmosphere, rivers and soils. Soils are known as resevoirs of pesticides, as from soil, pesticide may leach to other places such as air, plants, water and our food sources. These are based on various possibilities of interflow, runoff and movement of nutrients and pesticide from soils to plants and animals that are parts of human food chain (Muhammad *et al.,* 2014). Movement of pesticide from soil to groundwater sources are likely in tropical climate with interchange of raining and dry conditions. The water sources are infested by pesticide, as many incidents of pollution had been reported in developed countries. Early detection and protection of the ground and surface water will ensure a safer environment and cost saving, as treatment of pesticide polluted water are very costly (Brown *et al.,* 1995).

As for human being, the exposure to pesticide varies depending on its toxicity and persistence in the environment (OECD, 2001). Large number of people were reported to be affected by pesticide, with 3 million poisonings, about 750, 000 chronic diseases and 220 000 deaths each year worldwide (WHO, 2006). Most of the reports are from developing countries, yet, these developing countries only belong to one fifth of pesticide usage throughout the world. This figure is still vague as there are many unreported cases. Plantation workers, plantation personnel and consumers may be exposed to harmful pesticides through contaminated air, food and water with adverse effects such as headaches, dizziness, stroke, respiratory disorders, leukaemia, heart

attacks, cancer, brain and liver tumours and death (Paoletti & Pimentel, 2000; Alavanja *et al.,* 2004; Yousaf *et al.,* 2004; Tijani, 2006; Shou-zhen, 2007; Kedia & Palis, 2008).

Therefore, in this work, the monitoring study of lambda cyhalothrin in oil palm plantation was done to study the behaviour of lambda cyhalothrin in the environment. This study will look on the residue, fate and half life of lambda cyhalothrin in four agrosystem of palm oil including samples of leaves, fruits, soil and water. The finding obtained will add some knowledge on persistence of pyrethroid group insecticide, particularly lambda cyhalothrin, in creating a safer environment for living of mankind.

4.2 Methodology for Lambda Cyhalothrin

4.2.1 Lambda Cyhalothrin

Pesticide lambda cyhalothrin with brand name of Alert 2.8 EC and active ingredient content (a.i.) of 2.8% (w/w) was obtained from Hextar Chemicals Sdn. Bhd.

4.2.2 Area of Research

The field experiment was carried out at Amcorp Palm Oil Plantation (Figure 4.1). This plantation was located at Sepang, Selangor and it is about 700 metres from KLIA mosque, Headquarters of Malaysia Airports Holdings Berhad and KLIA police station (2°48'0.198" N,101°40'22.434 " E)(Figure 4.2).

Figure 4.1: Actual view of Amcorp Plantation at Sepang Selangor

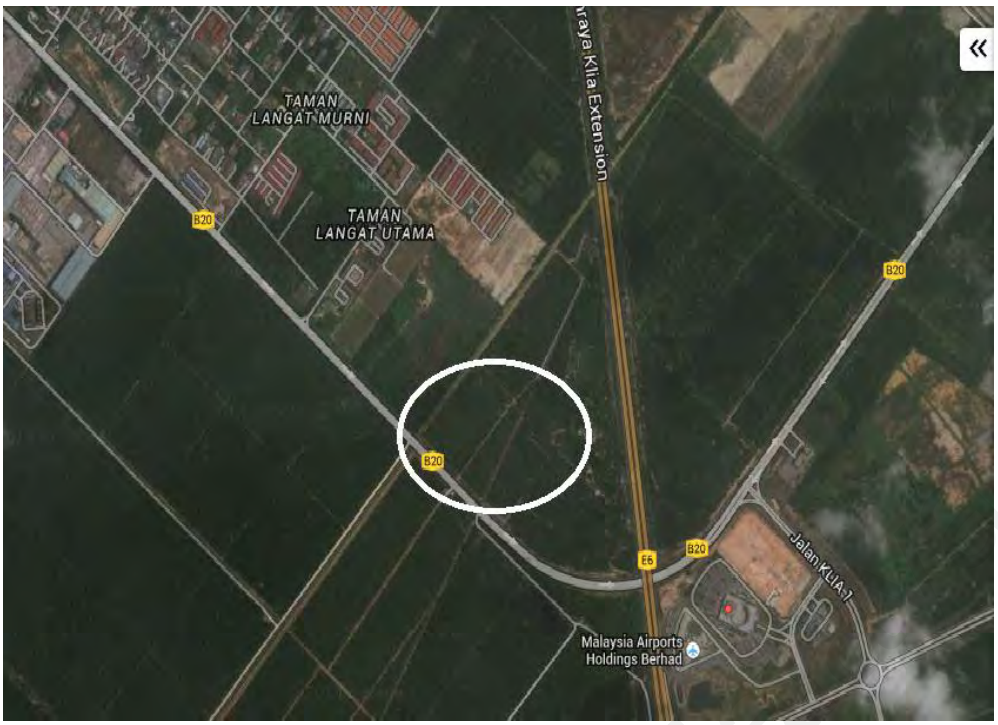

Figure 4.2: Map of Amcorp Plantation (in white circle) at Sepang Selangor
Sources: (Google Map)

4.2.3 Research Plot

Mobility study of lambda cyhalothrin was done in matured palm oil plantation with 4-5 years old oil palm. Area of studies was divided into 9 subplots. Each subplot contained 30 oil palm trees (Figure 4.3). There were three treatments, namely, untreated control (CR1, CR2, CR3), recommended dose treatments (1XR1, 1XR2, 1XR3) and double recommended dose treatments (2XR1, 2XR2, 2XR3) in three replicate. Untreated control was treated with water only. There were three replications for each treatment arranged in a randomized block design. Each subplot was separated with a buffer zone. Each subplot was tagged differently at the oil palm tree to indicate different treatments applied to it. Plot area was visualized in Figure 4.3.

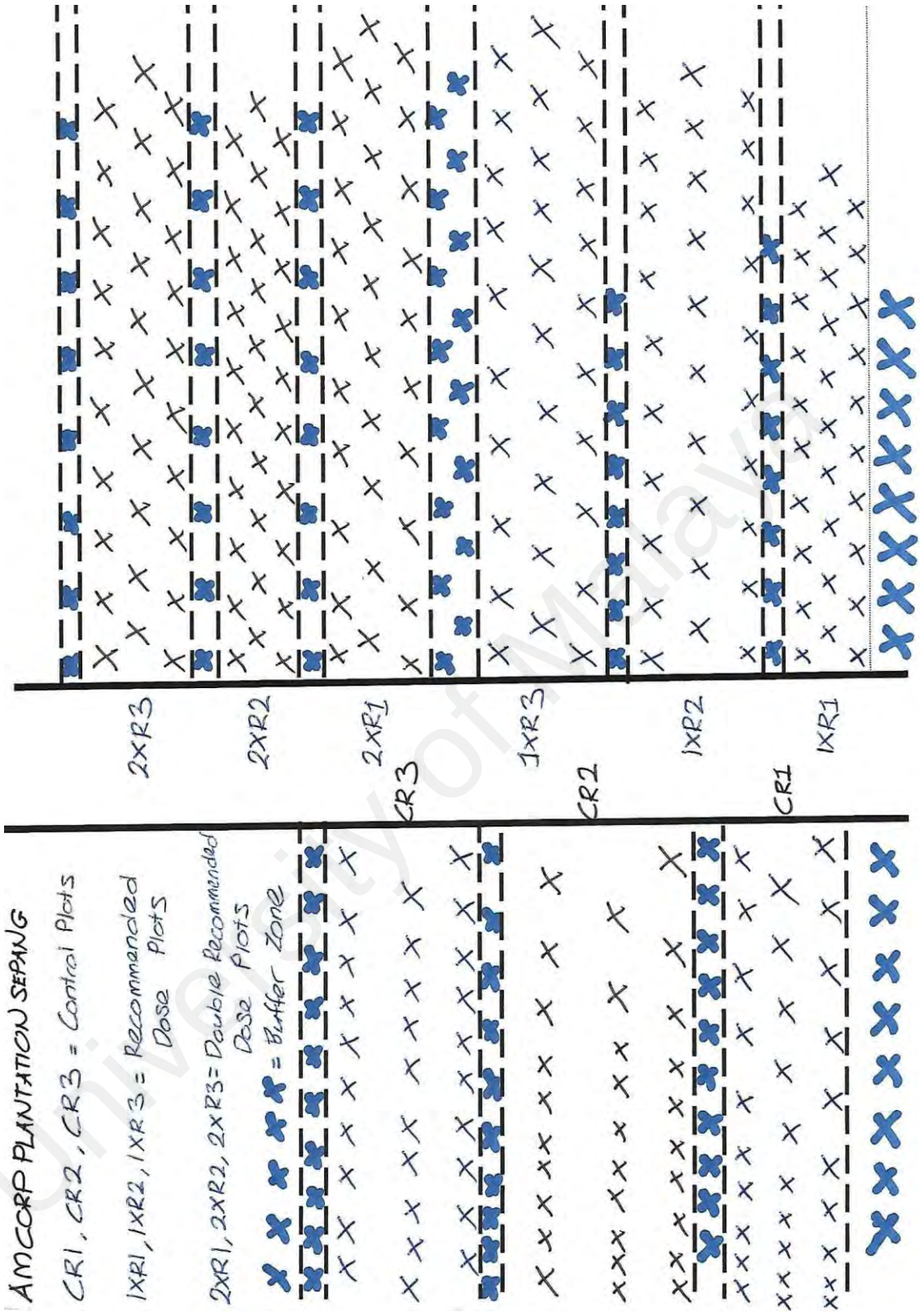

Figure 4.3: Design of actual plots on Amcorp Plantation at Sepang Selangor.

Each subplot was tagged with different tagging, indicating different dosage supplied according to untreated control (CR1, CR2, CR3), recommended dose treatments (1XR1, 1XR2, 1XR3) and double recommended dose treatments (2XR1,

2XR2, 2XR3). Figures 4.4 and 4.5 show different tagging applied to different oil palm trees according to different dosage.

Figure 4.4: Tagging apply to single recommended dosage plot (D1)

Figure 4.5: Tagging apply to double recommended dosage plot (K2)

4.2.4 Spraying of Lambda Cyhalothrin

The first application of lambda cyhalothrin (Alert 2.8 EC) at 31.5 and 63 g active ingredient a.i./ha was applied to the leaves of oil palm trees (Figure 4.6). In control

plots, only water was sprayed. 25 ml of lambda cyhalothrin pesticide was diluted in 10 litre of water. The volume of spray for lambda cyhalothrin is 180 liter of water per hectare (180 L/ha) and spraying was done on every side of the palm tree using a mist blower fitted with a hollow cone nozzle.

Figure 4.6: Spraying of Lambda cyhalothrin in Amcorp plantation

4.2.5 Sampling of Samples

Sampling for all samples was done before and after spraying of lambda cyhalothrin. All samples were collected on -1 (before spraying), 0 (Day of spraying) and 1, 3, 5, 7, 9, 10, 12, 14, 16, 18, 20, 29, 35 and 50 days. On the day of spraying (Day 0), sampling was done 6 hours after spraying. Table 4.1 shows sampling and spraying activities in each treatment plot.

Table 4.1: Activities in treatment plot

Days of sampling	Date	Activities
-1	5 March 2014	Sampling (fruits, soil, leaves and water) and plotting of sampling area
0	6 March 2014	Spraying of lambda cyhalothrin and sampling (6 hours after spraying)
1	7 March 2014	Sampling (fruits, soil, leaves and water)
3	9 March 2014	Sampling (fruits, soil, leaves and water)
5	11 March 2014	Sampling (fruits, soil, leaves and water)
7	13 March 2014	Sampling (fruits, soil, leaves and water)
9	15 March 2014	Sampling (fruits, soil, leaves and water)
10	16 March 2014	Sampling (fruits, soil, leaves and water)
12	18 March 2014	Sampling (fruits, soil, leaves and water)
14	20 March 2014	Sampling (fruits, soil, leaves and water)
16	22 March 2014	Sampling (fruits, soil, leaves and water)
18	24 March 2014	Sampling (fruits, soil, leaves and water)
20	26 March 2014	Sampling (fruits, soil, leaves and water)
29	29 March 2014	Sampling (fruits, soil, leaves and water)
35	4 April 2014	Sampling (fruits, soil, leaves and water)
50	19 April 2014	Sampling (fruits, soil, leaves and water)

4.2.5.1 Fruits (Crude Palm Oil and Palm Kernel Oil)

One bunch of matured fruits was sampled from each plot to make a total of three bunches of fruits for each recommended dosage, double recommended dosage and untreated plot. Then bunch which was chopped and fruits samples from the same plot (according to dosage treatment) were combined together to obtain homogenous samples. After that, fruits were further processed to obtain crude palm oil and palm kernel oil based on the methods of using mini hydraulic presser and soxhlet extraction.

4.2.5.2 Leaves

Fronds were sampled from each plot and composited. The leaves were obtained from top, mid and bottom sections of the palm fronds. The leaves were grounded, weighed in plastic bottles and stored at 4°C prior to analysis.

4.2.5.3 Soil

The physical and chemical properties of the soil were determined (Table 4.2). Soil samples were randomly collected at 5 points from each plot using auger from different depths (0-50 cm) (Figure 4.7). The soil sampling were according to five different depths: 0-10, 10-20, 20-30, 30-40 and 40-50 cm. And, the sampling was done from a distance of 0.5 meter surrounding the trees. After that, the soil samples collected from each point at the same depth were combined together.

Then, the soil samples were let to dry in room temperature for a week and sieved to remove large soil particles (Figure 4.8). Finally, all samples were stored at 4°C to prevent any activities of microorganism.

Figure 4.7: Collections of 5 different depths soil sample using auger

Figure 4.8: Sieving of soil samples

Table 4.2: Physical and chemical properties of soil

Properties	Results
Organic carbon (%) (n=4)	9.77
pH (n=5)	3.84
Cation exchange capacity (CEC) (n=6)	13.53
Particle density (n=4)	1.97
Bulk density (g/cm^3)	0.105
% Moisture content (% w/w) (n=19)	49.03
Water weight (n=19)	42.37
% Silt	28.01
% Clay	40.78
% Coarse sand	16.01
% Fine sand	15.20
Soil texture classification (Based on soil triangle)	Clay

4.2.5.4 Water

One liter of water sample was collected at two different groundwater sources (Figure 4.9) and stored at 4°C prior to analysis.

Figure 4.9: Water sources in Amcorp Plantation during March to April 2014.

4.2.6 Processing of Crude Palm Oil (CPO)

Oil palm fruit bunches were inserted into the autoclave for strerilization process. The fruit samples were then sterilized using the autoclave (Sakura Neoclave ASV-302) temperature of 120°C for 40 min. Sterilization was done according to sequences of untreated plot, samples with recommended dosage and samples of double recommended dosage. After sterilization, the samples were pressed using mini hydraulic presser manually to obtain the crude palm oil (Figure 4.10). After that, the crude palm oil was centrifudged at the speed of 2000 r.p.m to separate the oil from fibre and other sludges. Triplicate sample of crude palm oil were prepared and stored under temperature of -20°C before analysis.

Figure 4.10: Pressing of crude palm oil

4.2.7 Processing of Crude Palm Kernel Oil

In this work, the sterilized and pressed palm oil fruit were brought to MPOB Kluang for processing in purpose of separating the mesocarp from kernel of palm oil. The separation was done using a machine known as depalper (Figure 4.11). The kernel obtained from the separation process was left for a week for another process of separating the shell which was done from the flesh of palm oil kernel (Figure 4.12). Then, the flesh was blend using blender before being extracted using soxhlet extraction. The sample was extracted using N-hexane until all of the oil managed to be separated

well. N-hexane containing the oil underwent further process which are separated using rotary evaporator. Triplicate sample of crude palm kernel oil was prepared and stored under temperature of -20°C before analysis.

Figure 4.11: Separation using depalper machine

Figure 4.12: Palm kernel (left) separation from its mesocarp

4.3 Half life Determination for Lambda Cyhalothrin

Lambda cyhalothrin residues were determined by the analytical protocol described before this, and their log values over time (days after application) were subjected to weighted linear regression. The degradation rate constant and half-life were calculated using the first-order rate equation: $C_t=C_0-kt$, where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial deposits after application, and k is the degradation rate constant in days^{-1}. The half-life ($t_{1/2}$) is defined as the time required for the pesticide residue level to decrease to half the initial residue level after application and was calculated from the k value, being $t_{1/2}=\ln 2/k$ (Devi *et al.* 2014; Hoskins, 1966; Sharma *et al.* 2014).

4.4 Weather Conditions

Rainfall data along duration of studies were collected beginning from early of March 2014 to end of April 2014 (Figure 4.13 and 4.14). Spraying was conducted on the 6 March 2014, and the first samples were collected 6 hours after the initial spraying. The rainfall data during the experiment in Amcorp Plantation at Sepang Selangor are presented in Figure 4.13.

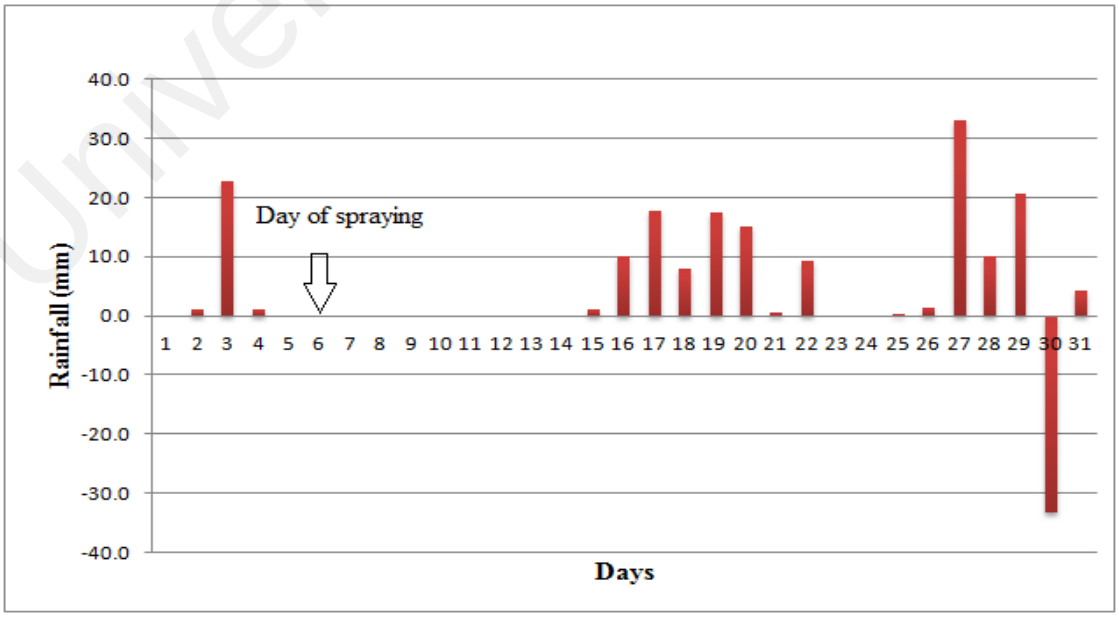

Figure 4.13: Quantity of rainfall in March 2014 at Amcorp Plantation of Sepang Selangor.

The highest rainfall recorded in month of March 2014 (Figure 4.13) was on 27 March 2014 with 33.2 mm while in a month of April (Figure 4.14),was 11 April 2014 with 65.4 mm. The frequency of rainfall in April was then compared to that in March 2013. This situation is normal where parts of Peninsular Malaysia encounters dry conditions with total monthly rainfall of less than 100 mm due to northeast monsoon period between November to the end of March. Therefore, dry conditions with less rain observed in month of March 2013 were due to this phenomena that occurs every year in Malaysia. After the monsoon season ends, there are changes in the trends, as observed in April 2014 with more frequent rain in that period of month (Malaysian Meterological Department (MMD), 2014). Table 4.3 indicates the weather information during the study period with information on average temperature (ºC), humidity (%) and rainfall data (mm).

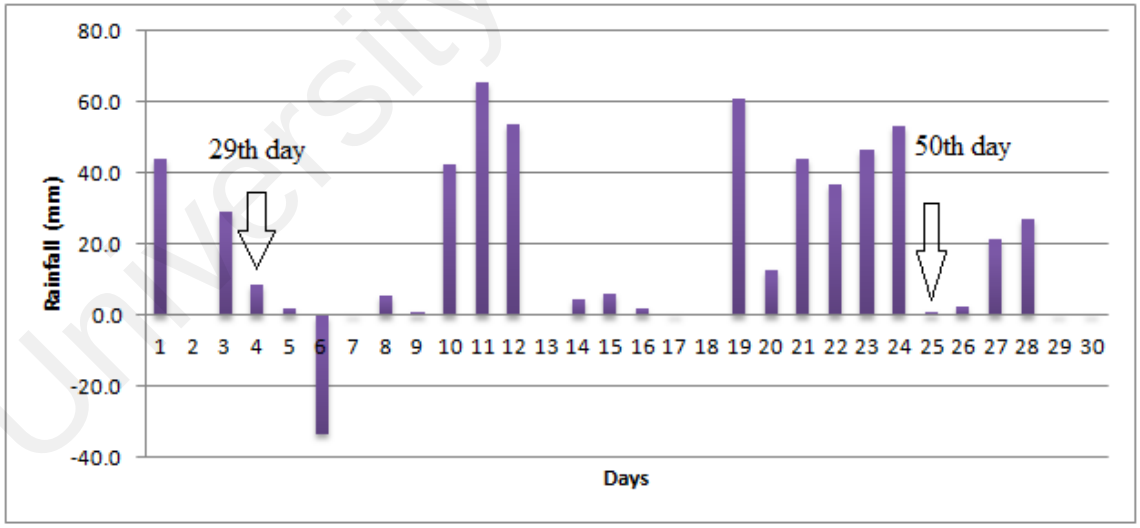

Figure 4.14: Quantity of rainfall in April 2014 at Amcorp Plantation of Sepang Selangor.

Table 4.3: Information on weather conditions during the field experimental period (Average temperature (ºC), average relative humidity (%), rainfall (mm))

Time (days)	Weather	Average Temperature (ºC)	Humidity (%)	Rainfall
0	Sunny	29.6 ºC	59.8 %	0.0 mm
1	Sunny	30.2 ºC	53.1 %	0.0 mm
3	Sunny	29.8 ºC	64.9 %,	0.0 mm
5	Sunny	30.2 ºC	48.8 %	0.0 mm
7	Sunny	29.8 ºC	67.6 %	0.0 mm
9	Little rain	29.2 ºC	68.3 %	1.2 mm
10	Moderate rain	27.3 ºC	83.1 %	10.0 mm
12	Little rain	26.1 ºC	89.2 %	8.0 mm
14	Moderate rain	26.6 ºC	88.8 %	15.2 mm
16	Little rain	29.1 ºC	73.3 %	9.4 mm
18	Sunny	30.5 ºC	55.7 %	0.0 mm
20	Little rain	29.1 ºC	75.1 %	1.4 mm

4.5 Results and Discussion

Table 4.4 and figure 4.15 illustrate the amount of lambda cyhalothrin residues in leaves of oil palm sprayed at recommended and double recommended dosage. Overall, it was observed that the residues were decreasing for both dosage with increasing days. The concentration of lambda cyhalothrin residues was decreasing according to increasing days after spraying until it was not detected on day 16 (recommended dosage) and day 20 (double recommended dosage) after spraying. The highest concentration of lambda cyhalothrin was found at day 0 (6 hours after spraying) for both dosage applied, and it is noted that the residues on spraying application at recommended dosage was lower compared to the double recommended dosage.

At recommended dosage, on day 0 (6 hours after spraying), the total concentration of lambda cyhalothrin in the foliar surfaces was 0.47 mg/kg. On day 1, the residue was reduced to 0.17 mg/kg with 63.83% lost from day 0 in less than 24 hours. On day 7, the total amount of lambda cyhalothrin dropped to 0.07 mg/kg with 85.11% loss from initial deposition at foliar surfaces. Eventually, on day 16 after the last spraying, lambda cyhalothrin was not detected at the foliar surfaces.

At double recommended dosage, on day 0 (6 hours after spraying), the total concentration of lambda cyhalothrin in the foliar surfaces was 0.83 mg/kg. On day 1, the residue was reduced to 0.22 mg/kg with about 73.50% less than initial amount sprayed. On day 9, the total concentration of lambda cyhalothrin decreased to 0.07 mg /kg with 91.57% reduced compared to early residue detection at day 0. Finally, the residues of lambda cyhalothrin were not detected on day 20 after spraying.

Table 4.4: Residue of lambda cyhalothrin in leaves after application at 31.5 g a.i./ha and 63 g a.i./ha

Days after application	Residues (mg/kg) (n=3)	
	31.5 g a.i./ha	63 g a.i./ha
Before spray	ND	ND
0	0.47 (-)	0.83(-)
1	0.17 (63.83)[a]	0.22 (73.50)[a]
3	0.10 (78.72)[a]	0.13 (84.34)[a]
5	0.09 (80.85)[a]	0.109 (86.87)[a]
7	0.07 (85.11)[a]	0.104 (87.50)[a]
9	0.06	0.07 (91.57)[a]
10	0.04	0.064
12	0.038	0.063
14	0.022	0.056
16	ND	0.051
18	ND	0.02
20	ND	ND
29	ND	ND

ND not detected

[a] Percentage dissipation of lambda cyhalothrin after spraying

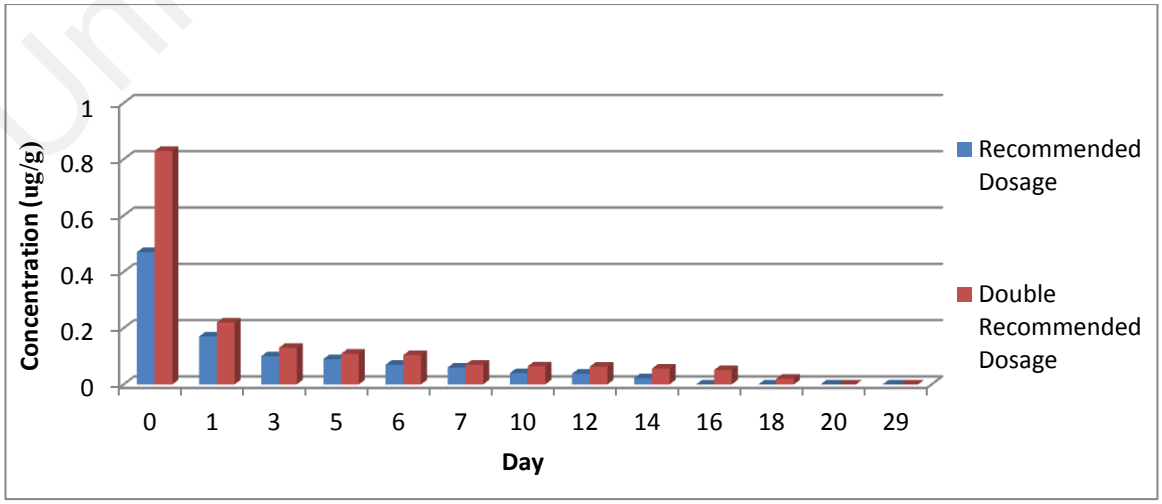

Figure 4.15: Residue of lambda cyhalothrin (µg/g) in leaves for day 0 until day 29 for both dosages applied

As stated earlier, the amount of lambda cyhalothrin residues detected in samples at applications of the recommended dosage was lower compared to that of the double recommended dosage. On day 0, the concentration of residues at the recommended dosage were about half from the double recommended dosage. The trends were not followed thereafter, with a variance of only 2 to 10 % between the two spraying dosage. Nevertheless, the results obtained indicated that the residue concentration at double recommended dosage was almost twice from the amount of residue at application of recommended dosage on day 12. Therefore, it is concluded that the dissipation of lambda cyhalothrin is independent of pesticide dosage, in term of the amount of residues obtained from variety of dosage applied.

The result obtained were analysed using analysis of variance (ANOVA) test to check whether there is potential difference among the two different dosages treatment towards the residue in palm oil leaves within day 0 until day 14 after spraying (Appendix 2). This method uses a single test to determine whether there is or is not a difference among the two treatments. In this single factor ANOVA procedure for recommended and double recommended dosage treatments, the null hypothesis H_o was of the form

$$H_o: \mu_R = \mu_{DR}$$

μ_R = mean residues of recommended dosage treatment

μ_{DR} = mean residues of double recommended dosage treatment

and the alternative hypothesis *Ha* was

Ha : there are different in the mean residue between the two different treatments

Based on the result obtained from the ANOVA tests, the null hypothesis H_o was accepted and concluded that there was no significant difference among the mean residues and hence, both recommended and double recommended dosage treatments

gave equivalent results, except for day 14. For day 14 after treatment, ANOVA test indicate that different dosage treatment between the two shows a significant difference.

This might be explained by the result of residues in day 12 and day 14 after spraying for recommended dosage treatment. Result shows a huge drop in the residues between those two days compared to residues in double recommended dosage treatment which show a steady drop in the residues amount. A lot of factors may contribute to this huge drop in residues such as environment factors and the plant structure itself which might contribute to result obtained. Therefore, the huge drop of residues between the two days consequently result in the significant different between the two treatments in day 14. Otherwise, for other days, we can concluded that there was no significant difference among the mean residues, thus showing that increasing the dosages of lambda cyhalothrin from 31.5 g a.i/ha to 63 g a.i/ha gave no significant difference.In other words, it is concluded that the dissipation of lambda cyhalothrin is independent of pesticide dosage, in term of the amount of residues obtained from variety of dosage applied.

The efficiency of spraying at recommended and double recommended dosage can be evaluated based on the concentration of lambda cyhalothrin remained in the foliar surfaces on day 0 compared to initial spraying concentration. This study has shown that the efficiency of spraying were only 0.27 % for recommended dosage and 0.24 % for double recommended dosage (Appendix 1).This is parallel with previous research indicating a lower percent of applied pesticide that may reach the targeted plant. It is reported that less than 0.3 % reach the targeted part whereas 99.7 % of it remains in the environment. Approximately 50 % of applied pesticide finally resulted as spray drift or runoff or moving to the ground as fallen leaves (Khan, 1980; Pimentel, 1995).

To the best of author's knowledge, only a few reports were conducted for residual behaviour of pesticide in the leaves of oil palm. Most of the reports were either done at oil palm nursery (Halimah & Tan, 2013; Maznah *et al.,* 2012) or oil palm plantation soil (Halimah *et al.,* 2005; Farahani *et al.,* 2008) etc. Halimah *et al.* (2012) reported that hexaconazole was detected in the leaves of oil palm until day 21 after treatment with soil drenching technique at the palms. Translocation via xylem or phloem are reported as major contributor. Carbendazim, the degradation product of benomyl was detected until day one after spraying, indicating a short dissipation of benomyl in nursery of oil palm plantation (Halimah & Tan, 2013).

Studies on thiram at oil palm nursery resulted in residues deposition in the soil at depth of 0-10 cm on day 0. Faster leaching of this pesticide, resulting in the residues found up to the depth of 50 cm on day 3 of spraying treatments and was undetectable thereafter. The residues were also found at samples of water and leaflet of oil palm on day 0 and day 1 of sampling. Sandy clay loam type of soil in the nursery showed lower adsorption towards thiram and wet season with rainfall almost everyday in the nursery were amongst the reason of detection in water and leaflet samples (Maznah *et al.*, 2012).

Behaviour of fluroxypyr-MHE and chlorpyrifos in the soil of oil palm plantations were studied and both pesticides were detected in the soil until day 5 at depth of 45 cm, and short persistence of both pesticide were due to soil properties and heavy rainfall during the study period (Halimah *et al.,* 2005; Halimah *et al.,* 2010). Halimah *et al.* (2013) also studied the behaviour of diuron in soil and found a moderate persistence of diuron up to day 60 after spraying treatments at all measurement depths (0-50 cm). Such moderate presistence were due to several factors including higher K_{oc}

value (soil organic carbon adsorption coefficients), low water solublity (K_{ow} = 42 mg/L at 25°C) and low organic matter content of treatment soil.

The results of previous studies on behaviour for variety of pesticides at oil palm plantations were in contrary with this study. The results of one study differed from another depending on various reasons. This indicated the need to focus on one pesticide or several pesticides from the same group of pesticide in order, to identify the exact behaviour in the plantation. Some indicates short persistence while another might indicate longer persistence in the environment. Therefore, there is a need to focus on each of them to look into its perseverance in the environment. As pesticides react differently in palm oil plantations, behaviour of lambda cyhalothrin in other plantations also shows various results (Banerjee *et al.* 2006, George *et al.,* 2013, Jayakrishanan *et al.* 2005). These are due to the differents nature of plantation and other factors.

Table 4.5: Lambda cyhalothrin in other matrices

Spraying of Lambda Cyhalothrin in Other Matrices	**Behaviour in matrices**	**Cause of dissipation**
Oolong Tea (Chen *et al.* 2012)	Either no change, reduce or increase in residue after processing steps	Processing steps after harvesting
Green & Black Tea (Seenivasan &Muraleedharan, 2009)	Decreasing in residue	Processing steps after harvesting
Cardamom (George *et al.* 2013)	Increasing in residue	Curing process after harvesting
Tomato (Jayakrishanan *et al.* 2005)	Decreasing in residue	Washing sample with water after harvesting
Grape (Banerjee *et al.* 2006)	Capsule suspension (CS) better than emulsifiable concentrate (EC)	Formulation of chemical pesticide

Beside environment and plant structures, there are also factor of pesticide formulation. Banerjee *et al.,* (2006) investigated the dissipation behaviour of lambda cyahlothrin in grape *(vitis vifera L.)* and also compared between emulsifiable concentrate (EC) and capsule suspension (CS) formulations for spraying of lambda cyhalothrin. Their finding indicated that the use of capsule suspension (CS) formulation was better in term of pest controls. The conclusion was made based on the initial residue deposits that are much higher and also faster dissipation based on DT_{50} value. But as overall, the rate of dissipation for both application showed almost similar trends with small different between the two.

Based on the results of this study, a short persistence of lambda cyhalothrin in leaves of oil palm plantations is based on several factors. One way is by focusing on the plant related aspects. On the plant itself, there are various aspects that can influence the behaviour of pesticide including plant age, age of the sample, stage development of the waxy cuticle in leaves, location of the leaves, leaves surface structure, density, height of the trees, physico-chemical properties of the compounds (e.g., vapour pressure, water solubilities and thus the Henry's law constants) and environmental conditions (e.g., temperature, humidity, moisture content, pH, uv radiation wind velocity and direction) (Lyman, 1990; Paterson *et al.,* 1994; Smith & MacHardy 1984; Hall *et al.,* 1997; Willis *et al.,* 1987).

Temperature of plantations also play a vital role in determining the persistency of lambda cyahlothrin. The temperature recorded in the plantation varied in the range of 25.6°C to 30.5°C (Table 4.13). This range of temperature can be considered moderate to high temperature in condition of typical tropical climate of Malaysia with the highest temperature recorded was 40.1°C at Chuping, Perlis on 1998 (MMD, 2014). Higher

temperature along the study period might also influence the dissipation half-lives in foliar surfaces. Some hypotheses made by researchers showed that higher temperature triggers microbial and chemical degradation in plants, therefore shortening their half lives (Willis & McDowell,1987; Stenersen, 2004; Yu *et al.,* 2006).

Eventhough rainfall is one of factor in determining the persistence of pesticide in Malaysian environment (Halimah *et al.,* 2005; Halimah *et al.*, 2010; Maznah *et al.,* 2012), but this factor was not considered in this study. This is because the reported rainfall in the months of spraying that was really low with no rain at day 0 until day 8 after spraying treatment, and only little rain on subsequent days (Figure 4.13 and 4.14**)**. However, the previous studies have shown that rainfall has a vital role in the leaching of pesticides from the foliar surfaces towards soil compartments and moving towards water sources (Leonard *et al.,* 1987; Willis, 1987; Halimah *et al,.* 2013; Zhang *et al.,* 2013).

The significant influence of pesticide dissipation on leaves surfaces are due to photodecomposition and ezymatic reaction. Sunlight and enzyme is a major pathway for pesticide degradation in the plant and it is more efficient than sources of dissipation in the soil (bacteria,sorption). Plant degradation through the action of enzyme was through biotic processes by plants and microorganisms, while photooxidation was reported as pathway for photodegradation by sunlight (Charles, 2004; Van Eerd *et al.,* 2003; Liu *et al.,* 2014). Both enzymatic transformation and photodegradation are influenced by high variable aspects such as substance vapor pressure, plant surface roughness and air temperature (Katagi, 2004; Hoagland *et al.,* 2001).

On the degradation pathways, one of degradation routes from plant tissues are through the action of enzymes while in the plant surface is through photodegradation. Thus, it is proposed that, these two routes were the major contributor to the faster

dissipation rate of lambda cyhalothrin in oil palm plantation.These routes were suggested as the major contributor of lambda cyhalothrin dissipation from leaves of oil palm due to several factors, that are, characteristics of lambda cyhalothrin, environmental conditions of plantation and analysis on dissipation result of lambda cyhalothrin in leaves of oil palm. Firstly, degradation of lambda cyhalothrin on plant tissue was suggested as direct spraying of pesticide to the foliar surfaces may cause substances rapidly being transported into the plant tissue instead of being degraded as deposit on plant surface (Charles, 2004).

Secondly, is based on the octanol / water-partition coefficient (K_{ow}) value. High octanol / water-partition coefficient (K_{ow}) causes the pesticide to be retained by fatty plant surface tissue and simultaneously causing slow mobility in the plant (log K_{ow} higher than 5 indicating higher value) (log K_{ow} of lambda cyhalothrin: 7.0) (Brooke, Dobbs & Williams, 1986). The slow mobility in the plant, consequently, causes the partitioning of pesticide into the inner plant cells, where enzymatic transformation occurs either through degradation or detoxification (Schonherr, 2002; Schreiber & Schoenherr, 1992).

Those previous studies on bromopropylate and alpha-cypermethrin (log K_{ow}= 5.2 & 5.4) indicates the high tendencies of this pesticides towards foliar surfaces and subsequently potential of penetrating the inner part of plant cells (Charles, 2004; Fujisawa *et al.,* 2002). Lambda cyhalothrin with octanol / water-partition coefficient (K_{ow}) value of 7.0, results in a big potential for lambda cyhalothrin to be retained by plant surface, thus partitioning into the plant cell to be degraded through enzymatic reaction (Amit *et al.,* 2010).

Futhermore, those lipophilic compunds like lambda cyhalothrin tend to accumulate by epicuticular wax of plant leaves through the action of long-chain

polyesters (Reischl *et al.,* 1989; Calamari *et al.,* 1991). The nonabsorbed active ingredients can progress in two ways, either going through into the plant tissues from the epicuticular waxes or remain on the surface of the leaves. If it remains on surface, it may be redissolved into guttation water or morning dew water and be available to insect (Bonmatin *et al.,* 2014). But, if penetration happen, degradation process might continue after that.

Volatilisation may be one of dissipation pathway from the plant surface. Despite windy conditions, substance-specific vapor pressure influences the volatilisation process. Pesticide with high value of vapor pressure may tend to volatile faster compared to low vapor pressure pesticide. But, the low vapor pressure of lambda cyhalothrin with approximately 1.4×10^{-9} mmHg, indicating a slower volatilisation from the plant surfaces and a slightly more time for lambda cyhalothrin to accumulate into the plant tissues (Amit, 2010; Spynu, 1989; McCrady & Maggard, 1993; Charles, 2004; Katagi, 2004). Therefore, it is suggested that, one of the pathway for lambda cyhalothrin degradation in the leaves of oil palm was by enzymatic reaction caused by the penetration of lambda cyhalothrin into the plant tissue from its surfaces.

Another major pathway suggested for lambda cyhalothrin degradation was through photodegradation at the plant surfaces. Photodegradation is a faster process compared to enzyme and bacteria action (Marcheterre *et al.,* 1988). The persistence of lambda cyhalothrin up to day 14 and 18 for recommended and double recommended spraying at the plantation may be explained by degradation through the plant tissue. The faster degradation rates when comparing to the earlier part of sample collections from day 0 up to day 9 may be through photodegradation.

This was supported with evident that environmental biodegradation through an action of enzyme and bacteria is slow, and only certain specific bacteria can degrade

pyrethroids group including lambda cyahlothrin in the natural environment. As an example, biodegradation half-lives of six types of pyrethroids were between 30 and 131 hours and after 15 days, only 35 % of fenpropathrin (FENP), one of pyrethroid group chemicals, degraded by an appropriate bacterial strain (Lee *et al.*, 2004; Zhang *et al.,* 2009).

Photochemical reaction are very common to happen for pyrethroid groups. Pesticides mostly showing a UV-vis absorption bands at lower wavelength. Natural sunlight that penetrates the earth atmosphere are in the range of 290 nm to 800 nm. The intensity of sunlight weakeaned as its enter the atmosphere through aerosol and molecular scattering and by absorption of atmospheric gases (Yeasmin, 2006). One study investigated the behaviour of pyrethroid group with light that mimic the sunlight with UV and sunlight irradation. They discover that, pyrethroid molecules contain double bonds that are unstable to light, thus, proposing photooxidation degradation as the major reaction in the pyrethroid (Liu *et al.,* 2014). Sunlight or uv light (> 290 nm), when interact with pyrethroid group will led to the formations of photoproducts that happen due to some cleavage at structure of pyrethroid group chemicals. The three possible spectrums that mostly involved in degradation of pyrethroid group are UV spectrum with are UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm). In atmosphere, ozone have the ability to absorb almost all photons in between the regions of 280 and 320 nm, thus making the intensity of UV-B radiation decreases sharply at earth surfaces. While, stratospheric ozone absorbs the uv light at shorter wavelength of 220-290 nm thus, simultaneously absorbing photons in the UV-C region. The least biologicallly harmful region, UV-A (320-400 nm) does manage to penetrate the earth surface as this UV light is not absorbed significantly by 0_2, ozone or other components of atmosphere (Yeasmin, 2006). The fact that chemical bond energy of pyrethroid were generally below 380 nm, causing the higher absorption of pyrethroid

group chemicals towards the UV-A (320-400 nm). This lead to the cleavage of chemical bonds in pyrethroid group structure and turning it to its byproducts.

Some of studies done by Holmstead *et al.* (1978) indicated that the isomers undergoing isomerization of cyclopropane ring when irradiated with UV light (> 290 nm) or sunlight. Deltamethrin (1R, cis, αR) in hexane, one of pesticide belong to pyrethroid group, was chosen to be test under bright summer sunlight for 5 days. The observation showed the formation of 1S-cis-αS, 1R-trans-αS and 1S-trans-αR. Negative results was found happened with no reaction occured when sample were stored in hexane in the dark places. They concluded that isomerization might be due to the light, but at the same time they also agreed to the finding that, solvents can also trigger the epimerization (Leicht *et al.,*1996; Maund, 2002). According to Fernandez-Alvarez *et al.* (2007), the study was on the photodegradation of lambda cyhalothrin under UV light (18 W, 254 nm) for 20 minutes. They discovered that, 95% of initial amounts supplied were degraded with first-order rate constant (k_{ap}) and half-life ($t_{1/2}$) were determined to be 0.163 min^{-1} and 4.26 min^{-1}, respectively.

Based on this research, it is demonstrated that the rapid deterioration of lambda cyhalothrin in the presence of natural sunlight. For example, on day 1, the residues were reduced to 63.83% and 73.50% for recommended and double recommended dosage from day 0 in less than 24 hours. While, 80.85% and 86.87% lost from the initial deposition on day 0, for recommended and double recommended dosage at day 5 after spraying application. This showed a strong evident of rapid degradation of lambda cyhalothrin in between day 0 to day 9 and photodegradation was proposed as the degradation mechanism of lambda cyhalothrin in leaves of oil palm. Therefore, photodegradation and enzymatic reaction were suggested as a major pathway in degradation of lambda cyhalothrin. This is based on the results of less than 20 days for

lambda cyhalothrin to be undetectable in the leaves and the high percentage of lost starting from day 0 to day 9.

4.5.1 Half life of Lambda Cyhalothrin in Leaves

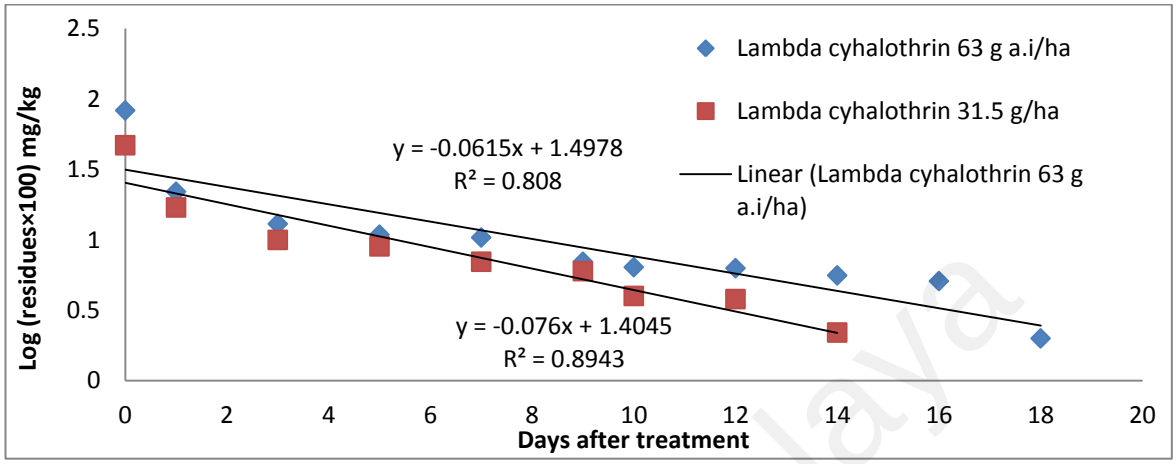

Figure 4.16: Dissipation curves of lambda cyhalothrin residues in leaves of oil palm plantation

The degradation kinetics of lambda cyhalothrin in leaves of oil palm was determined by plotting residue concentration (*C*) against time, and the maximum squares of correlation coefficients found were used to determine the equations of best fit curves. Confirmation of the order of kinetics was further made graphically from the linearity of the plots of log *C* against time. It was observed that the total lambda cyhalothrin residues did not follow the first-order kinetics (Figure 4.16). However, these residues followed the pseudo-first-order-kinetics with a coefficient of determination (R^2) value of 0.808 and 0.8943 for recommended and double recommended dosage, respectively. The regression equation for the recommended dosage of lambda cyhalothrin was y = −0.076x+1.4045, and for lambda cyhalothrin at 63 g a.i./ha, the equation was y = −0.0615x+1.4978 with half-life values of approximately 3.96 and 4.88 days respectively.

There might be different when comparing data from this studies from those elsewhere eventhough for the same pesticide in the same type of plant. Different type of

crops, non identical plant surface properties with different environmental conditions (temperature, humidity, UV irradiation) make the half life different from one study to another (Loutfy *et al.,* 2014). But, there are possibilities of pesticide from the same group or classes showing a similar trends in its half life (Fantke *et al.,* 2014).

George *et al.* (2013), also observed the half life values in the ranges of 4.40-4.55 days for lambda cyhalothrin in cardomom following application of lambda cyhalothrin at 0.0025 %. Similarly, Barik *et al.* (2010) reported that the half life of lambda cyhalothrin was 4.8 days following a 'readymix' formulation at 66 g. a.i./ha in transplanted paddy and Seenivasan & Muraleedharan (2009) found that the half life value of lambda cyhalothrin in tea were in the range of 2.8 to 3.5 days following three different applications at 250, 500 and 1000 ml/ha, respectively. Those results indicate faster dissipation of lambda cyahlothrin in variety of matrices.

4.5.2 Risk assessment of lambda cyhalothrin on leaves of oil palm

The increase in the use of oil palm leaves especially as functional food has cause great concern among the consumers on harmful effects of pesticide in food. Residue implication of foliar application for lambda cyhalothrin has been evaluated by comparing theoretical maximum residue contribution (TMRC) of the pesticide with its maximum permissible intake (MPI). The prescribed acceptable daily intake (ADI) of lambda cyhalothrin is 0.02 mg/kg/body weight /day (WHO, 2003). Multiplying the ADI with the average body weight of an Malaysian adult taken as 62.65 kg (Tan *et al.*, 2012), the MPI was found to be 1.253 mg/person/day. The TMRC has been calculated by multiplying the maximum residue levels with average per capita daily consumption of 78 g of vegetable in Malaysian context (FAOSTAT, 2015). Based on lambda cyhalothrin residue field trial on oil palm tree, data reflecting maximum residues that may occur under recommended dose (31.5 g a.i./ha), and worst condition on double recommended dosage (63 g a.i./ha), the TMRC values on 0 day were found to be 0.037

and 0.065 mg /person/day. Even at worst condition, the value of TMRC was still below the value of 1.253 mg /person /day for MPI. Therefore, it is safe to use lambda cyhalothrin for foliar application at recommended dosage, for consumer consumption of both the leaves and also palm oil. Thus, one day is suggested as a safe waiting period before any consumer consumption.

4.5.3 Residue of Lambda Cyhalothrin in Soil

Residue of lambda cyhalothrin in soil of all plot was detected at spraying treatments of double recommended dosage. Except that, detection was only on the earlier part of spraying (day 0 to day 3) and on the upper part of soil compartments (0 to 20 cm). Over time, the residue in upper part of soil begin to decrease. But, it needs to be emphasis here that detection is less than its limit of detection (LOD) of 0.002 µg/g. This show that the detection is really low and can be declared as free or no detection of lambda cyhalothrin residue in soil sample.

Previous studies provided guidance in exploring the dissipation process that may happen in the field soil. Despite having simulated rainfall at 300 mm, Rani *et al.* (2014) only detected the presence of cypermethrin at the top 15 cm. Almost similar studies were done by Chai & Zaidel (2011) in three types of soils for cypermethrin and it was found that cypermethrin remained in the top 0–10 cm layer of the three soils. Meanwhile, Tariq *et al.* (2006) observed the highest concentrations of lambda cyhalothrin in the top 0–10 cm layer. This indicated the low mobility of pyrethroid group pesticide on the field soil, hence low possibility for its contamination in the ground water. The lower mobility of lambda cyhalothrin in the soil structure makes it present in the upper part of soil. Hill *et al.* (1985) indicated that the rapid initial residue decline (0 to 2 weeks) were caused by initial surface losses (evaporation, photolysis, chemical hydrolysis and physical loss) combined with microbial degradation.

Previous studied reported that dissipation of pyrethroids is mostly microbial (Kaufmann *et al.* 1977; Williams, 1979; Chapman, 1981). The soil microbial activities increases with increasing soil temperatures up to a maximum level of 30°C to 35°C. Biodegradation was the suggested route for dissipation of lambda cyhalothrin in the soil, as observed by the rapid loss of lambda cyhalothrin in non-sterile soil compared to sterile soil in laboratory studies (European Commission, 2001). Hydroxylation and subsequent cleavage of ester linkage derived a two main degradation products, which further were converted into carbon dioxide that are preferred routes of lambda cyhalothrin degradation. Twenty two to eighty two days were the initial half lives suggested with lambda cyhalothrin to be strongly adsorbed on soil particles (International Programme on Chemical Safety, 1990)

4.5.4 Residue of Lambda Cyhalothrin in Water

Water sample was collected at two natural groundwater sources in the plantation. As reported earlier, the condition of climate on the previous part of spraying was really dry with zero amount of water (mm) in the drainage system (Figure 4.17). This dry condition enabled our early expectation of none residue to be detected in all plots involved in this study to be true. There is no detection on water sample collected from the ground sources.

Figure 4.17: Dry drainage system in Amcorp Plantation during March to April 2014.

Based on previous studies, it is known that preferential flow phenomena are the main contributors towards faster movement of pesticide to drainage system (Kladivko *et al.,* 1991; Novak *et al.,* 2001; Neumann *et al.,* 2002; Gardenas *et al.,* 2006). Meanwhile, pesticide pollution of ground and surface water is an issue in tropical countries, with heavy rainfall, high humidity and high temperature (Halimah *et al.,* 2010; Cheah *et al.,* 2001). Therefore, different outcome was expected, if the normal climate condition of Malaysia occurred during period of spraying treatments and sample colection afterwards. This is due to pyrethroid that behave differently in different water sources depending on the pH, rainfall and aquatic system in the water system.

4.5.5 Residue of Lambda Cyhalothrin in Crude Palm and Palm Kernel Oil

Analysis on crude palm oil (CPO) and kernel oil (CPKO) were carried out for all plots. However, lambda cyhalothrin was not detected in all sample collected and processed from day 0 to day 50 of sampling. Therefore, the use of lambda cyhalothrin in palm oil plantation is safe eventhough with dosage was more that what was recommended.

4.6 Conclusion

Dissipation behaviour of lambda cyhalothrin in samples of oil palm plantation shows different results with initial detection of 0.47 and 0.83 mg/kg, following spraying application at 31.5 and 63 a.i./ha on leaves of palm oil and no residual detection in other three samples. Quick dissipation during the first five days after spraying application, followed by a more gradual dissipation after that on the leaves of palm oil. The detection lasted until day 14 and day 18 respectively, with half life of approximately 3.96 and 4.88 days for recommended and double recommended dosage. As for risk assesment data, the lower theoretical maximum residue contribution (TMRC) value compared to MPI, indicating it is safe for consumer consumption of both leaves and oil

palm fruits. A pre harvest interval (PHI) of one day has been recommended between the last application of lambda cyhalothrin with frond and oil palm fruit collections.

CHAPTER 5: CONCLUSION

5.1 Research Conclusion

The use of lambda cyhalothrin continuously as insecticide in oil palm plantation may result in contaminant of lambda cyhalothrin residues in air, soil, water, groundwater, sediments and crops such as fruits and vegetables. Therefore, in ensuring that our food sources and environments are free from this pesticide, a method of pesticide detection need to be developed and validated to detect this pesticide in those samples. Knowing the residues present in samples will help in understanding the nature of pesticide, thus, starting an effort to either remove or prevent the contamination of pesticide in the environment and food sources. Steps such as developing a bio-pesticide, improving in the nature and behaviour of pesticide, for example, faster disispation of pesticide in environment, as well as faster action towards the targetted pest, are some of the effort that can be done to ensure the pesticide are more environmental friendly.

The method for lambda cyhalothrin detection on palm oil and agroenvironment samples had been developed before, and this method need to be verified and validated prior being implemented by other researchers. Verifications of method developed are important in promising a valuable data for other analysts when they want to used those new made methods. In this study, five general verifications were done on established method for detection of lambda cyhalothrin in palm oil plantation including method of detection in crude palm oil and palm kernel oil, soil, leaf and water samples. These include identification and confirmation for analyte of interest, linearity, selectivity, recovery and detection limit of instrument.

The first study focused on the identification and confirmation of lambda cyhalothrin using gas chromatography - mass spectrometer (GC-MS). It is important to identify this specific compund especially in monitoring study where variety of matrices are involved in the samples. Lambda cyhalothrin was confirmed by the detection of mass detector with the base peak at m/z = 181 followed by 197 and 208.

Next, linearity study was done to test on the ability of method developed which produced results that were equivalent to the analyte concentration in sample. Usually, linearity test is done in several working ranges that enables analyst to create a calibration curves for compound involved. The results of linearity test for lambda cyhalothrin show that the method developed are quantitive and adequate for analysis with the coefficient of correlation (R^2) for the calibration curves was 0.994.

Selectivity refers to the ability of analyte of interest (lambda cyhalothrin) to differentiate from other responses or inteferences. This can be done by developing a chromatogram of analyte of interest in its pure form and compared it with sample matrices. Based on chromatogram, lambda cyhalothrin was able to differentiate from the four samples of crude palm oil, leaf, soil and water, thus, displaying the method of lambda cyhalothrin extraction and clean up step involved on some of the methods, which is selective method.

Verification studies was further strengthen by studies on limit of detection (LOD) and limit of quantification (LOQ). These two parameters were important to the researchers on the ability and limit of their instrument towards analyte of interest. For this studies, method used in determining limit of detection (LOD) and limit of quantitation (LOQ) were based on signal to noise ratio (S/N). Limit of detection (LOD) for lambda cyhalothrin is 0.002 μg/g and 0.02 μg/g for its limit of quantification (LOQ). Final test on validation study in this research involved accuracy or recovery of method. This study was done to measure the closeness of analyte in sample to its true value. Recovery studies for four samples were done in three different ranges of low, medium and high concentrations. The mean recoveries of lambda cyhalothrin were found to be

86.5-90.4 %, 96.1-108.8 %, 80.6-105.2 % and 83.25-90.12 % respectively, for crude palm and palm kernel oil, leaves, soil and water sample at three level of concentrations. This result with low relative standard deviation values show the good performances of the method developed. Therefore, method that have been developed and verified were used to detect the residue and looking on the behaviour of lambda cyhalothrin in Malaysian palm oil plantation. Spraying of lambda cyhalothrin was done according to the recommended and double recommended dosage, and all the four samples were collected on different days.

Results of lambda cyhalothrin spraying in palm oil plantation showed that the residue of lambda cyhalothrin remained in the leaf or frond of oil palm until day 14 and day 18 according to spraying based on recommended and double recommended dosage. Photodegradation and enzymatic reaction were suggested pathway for degradation of lambda cyhalothrin from leaf of oil palm. The initial deposition of lambda cyhalothrin on leaf of oil palm were recorded as 0.47 and 0.83 mg/kg, six hours after spraying on day 0, with low efficiency of spraying with just 0.27% and 0.24% based on comparison of deposition of lambda cyhalothrin on day 0 and intial spraying concentrations. Calculation on half life shows approximately 3.96 and 4.88 days for spraying at recommended and double recommended dosage on leaf of oil palm.

On other samples including soil, water, crude palm oil and crude palm kernel oil, it is recorded that, there are no detection of lambda cyhalothrin in any of these samples. No detection on other samples were based on various reasons including the dry condition of plantation, faster degradation of lambda cyhalothrin due to sunlight exposure, lower mobility of lambda cyhalothrin in soil compartment and oil palm. Risk assessment data indicated safe consumption of leaf and palm oil and a preharvest interval (PHI) of one day has been recommended for lambda cyhalothrin spraying at palm oil plantation.

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APPENDIX

Appendix 1

Calculation on Efficiency of Spraying

Recommended Dosage:

Lambda cyhalothrin at application of 31.5 g a.i/ha and spray voume of 180 L/ha

$$\frac{0.0315 \text{ kg a.i/ha}}{180 \text{ L/ha}} \times 100\ \%$$

$= 0.0175\ \%$

Residue on leaf at day 0: 0.47 ppm

$0.47 \text{ ppm} \times 10^{-6} \times 100\ \%$

$= 4.74 \times 10^{-5}\ \%$

Efficiency of spraying for recommended dosage:

$$\frac{4.74 \times 10^{-5}\ \%}{0.0175\ \%} \times 100\ \%$$

$= 0.27\ \%$

Double Recommended Dosage:

Lambda cyhalothrin at application of 63 g a.i/ha and spray voume of 180 L/ha

$$\frac{0.063 \text{ kg a.i/ha}}{180 \text{ L/ha}} \times 100\ \%$$

$= 0.035\ \%$

Residue on leaf at day 0: 0.83 ppm

$0.83 \text{ ppm} \times 10^{-6} \times 100\ \%$

$= 8.30 \times 10^{-5}\ \%$

Efficiency of spraying for double recommended dosage:

$$\frac{8.30 \times 10^{-5}\ \%}{0.035\ \%} \times 100\ \%$$

$= 0.24\ \%$

Appendix 2

Table 1: ANOVA test for different dosage treatment for lambda cyhalothrin at day 0, n=3

From the ANOVA test, the calculated P-value was 0.055742. Since this value was greater than the significance level at 0.05, the null hypothesis *Ho* was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	1.422	0.474	0.004288
Double Recommended	3	4.987	0.831167	0.06635

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.255136	0.255136	5.247757	0.055742	5.591448
Within Groups	0.340327	0.048618			
Total	0.595463				

Table 2: ANOVA test for different dosage treatment for lambda cyhalothrin at day 1, n=3

From the ANOVA test, the calculated P-value was 0.317964. Since this value was greater than the significance level at 0.05, the null hypothesis *Ho* was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	1.0123	0.168717	0.007794
Double Recommended	3	0.8814	0.22035	0.002035

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.006398	0.006398	1.135559	0.317694	5.317655
Within Groups	0.045077	0.005635			
Total	0.051475				

Table 3: ANOVA test for different dosage treatment for lambda cyhalothrin at day 3, n=3

From the ANOVA test, the calculated P-value was 0.163365. Since this value was greater than the significance level at 0.05, the null hypothesis *Ho* was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.2985	0.0995	0.000595
Double Recommended	3	0.52	0.13	0.0006

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.001595	0.001595	2.667192	0.163365	6.607891
Within Groups	0.00299	0.00598			
Total	0.004584				

Table 4: ANOVA test for different dosage treatment for lambda cyhalothrin at day 5, n=3

From the ANOVA test, the calculated P-value was 0.508542. Since this value was greater than the significance level at 0.05, the null hypothesis *Ho* was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.287	0.095667	0.00108
Double Recommended	3	0.437	0.10925	0.000321

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.000316	0.000316	0.506333	0.508542	6.607891
Within Groups	0.003123	0.000625			
Total	0.00344				

Table 5: ANOVA test for different dosage treatment for lambda cyhalothrin at day 7, n=3

From the ANOVA test, the calculated P-value was 0.559922. Since this value was greater than the significance level at 0.05, the null hypothesis *Ho* was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.2858	0.07145	0.007237
Double Recommended	3	0.311	0.103667	0.000564

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.001779	0.001779	0.389515	0.559922	6.607891
Within Groups	0.02284	0.004568			
Total	0.02461				

Table 6: ANOVA test for different dosage treatment for lambda cyhalothrin at day 9, n=3

From the ANOVA test, the calculated P-value was 0.745797. Since this value was greater than the significance level at 0.05, the null hypothesis *Ho* was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.191	0.063667	8.63E-05
Double Recommended	3	0.198	0.066	0.000049

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	8.17E-06	8.17E-06	0.12069	0.745797	7.708647
Within Groups	0.000271	6.77E-05			
Total	0.000279				

Table 7: ANOVA test for different dosage treatment for lambda cyhalothrin at day 10, n=3

From the ANOVA test, the calculated P-value was 0.558515. Since this value was greater than the significance level at 0.05, the null hypothesis Ho was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.2764	0.046067	0.001037
Double Recommended	3	0.1805	0.060167	0.001097

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.000398	0.000398	0.377229	0.558515	5.591448
Within Groups	0.007378	0.001054			
Total	0.007776				

Table 8: ANOVA test for different dosage treatment for lambda cyhalothrin at day 12, n=3

From the ANOVA test, the calculated P-value was 0.225557. Since this value was greater than the significance level at 0.05, the null hypothesis Ho was accepted and concluded that there was no significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.113	0.037667	1.43E-05
Double Recommended	3	0.1894	0.063133	0.000935

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.000973	0.000973	2.049034	0.225557	7.708647
Within Groups	0.001899	0.000475			
Total	0.002872				

Table 9: ANOVA test for different dosage treatment for lambda cyhalothrin at day 14, n=3

From the ANOVA test, the calculated P-value was 6.79E-05. Since this value was less than the significance level at 0.05, the null hypothesis Ho was rejected and concluded that there was significant difference among the mean residues for both treatments.

SUMMARY

Dosage treatment	No. of measurement	Sum	Average	Variance
Recommended	3	0.1342	0.022367	6.33E-06
Double Recommended	3	0.222	0.0555	0.000114

ANOVA

Source of Variation	Sum of Squares (SS)	Mean Squares (MS)	F (Calculated)	P-value	F (critical)
Between Groups	0.002635	0.002635	56.56526	6.79E-05	5.317655
Within Groups	0.000373	4.66E-05			
Total	0.003007				