The Dissipation of Profenofos, λ-Cyhalothrin and Chlorothalonil in Vegetable and Soil under Humid Tropical Condition

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Declaration

I hereby declare that no portion of work referred to in this dissertation has been submitted of an application for another degree of qualifications of this or any other university or institutions of higher learning.

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ABSTRACT

The dissipation dynamic of 3 pesticides, namely profenofos, λ-cyhalothrin and chlorothalonil in green mustard and soil was studied. Field experiments were conducted in dry and wet season under net house and open field to determine the dissipation rate, half-life ($t_{1/2}$) and pre-harvest interval (PHI) of these pesticides in green mustard and soil. The green mustard was treated with commercial profenofos (ELAK 45EC), λ-cyhalothrin (ALERT 2.8EC) and chlorothalonil (Daconil 2787) at recommended dosage following good agricultural practice. Residue of pesticides in green mustard and soil were determined after the second pesticides application over a period of 22 days. Dissipation of all 3 pesticides in green mustard was fitted the pseudo first order kinetic. Calculated half-lives of profenofos, λ-cyhalothrin and chlorothalonil in green mustard during dry and wet season were 0.66–0.72 day and 1.28–1.76 days; 1.18–1.25 days and 2.51–3.69 days; 0.93–1.07 days and 2.01–2.21 days, respectively. In incubated soil, calculated half-life was 24 days, 37 days, and 15.2 days for profenofos, λ-cyhalothrin and chlorothalonil respectively. The 3 pesticides dissipation in both green mustard and soil followed biphasic dissipation pattern, with faster dissipation in phase I (0–3 days) and manifesting slower rate of dissipation in phase II (3–22 days). Results obtained from this study showed pesticides' physicochemical properties, cultivation systems used and climatic factors such as sunlight radiation, surrounding temperature and rainfall influenced the dissipation rate of pesticides. The dissipation rate of pesticides in vegetable and soil under humid tropical climate was found to be faster compare to temperate and subtropical region with lower surrounding temperature. λ-cyhalothrin residue dissipated below tolerance level after 3 days. However, longer pre-harvest interval of 14–22 days was required for profenofos and chlorothalonil to comply with the national tolerance level of 0.01 mg kg^{-1}. The long waiting period suggested that repeated application of profenofos and chlorothalonil onto green mustard is not recommended 14 days prior to harvest.

Pelesapan Profenofos, λ-Cyhalothrin dan Chlorothalonil dari Sayuran dan Tanah di Persekitaran Beriklim Tropika

ABSTRAK

Dinamik pelesapan profenofos, λ-cyhalothrin dan chlorothalonil dari sawi hijau dan tanah telah dikaji. Kajian lapangan dijalankan di dalam sistem penanaman kelambu dan penanaman terbuka pada musim kering dan musim hujan bagi menentukan kadar pelesapan, separuh hayat ($t_{1/2}$) dan tempoh masa optimum untuk penuaian. Sawi hijau disembur dengan racun perosak komersial profenofos (ELAK 45EC), λ-cyhalothrin (ALERT 2.8EC) dan chlorothalonil (Daconil 2787) pada kadar serta dos yang ditetapkan oleh Skim Amalan Ladang. Residu mula ditentukan selama 22 hari selepas semburan kedua. Separuh hayat bagi profenofos, λ-cyhalothrin dan chlorothalonil di dalam sawi hijau pada musim kering dan hujan adalah 0.66–0.72 hari dan 1.28–1.76 hari; 1.18–1.25 hari dan 2.51–3.69 hari; 0.93–1.07 hari dan 2.01–2.21 hari. Proses pelesapan profenofos, λ-cyhalothrin dan chlorothalonil dari sawi hijau dan tanah terdiri daripada 2 fasa, dimana fasa I (hari 0-3) kadar pelesapan adalah tinggi manakala bagi fasa II (hari 3-22) kadar pelesapan adalah lebih perlahan. Hasil kajian ini menunjukkan kesan sifat fisikokimia racun perosak, sistem penanaman serta faktor iklim tempatan seperti radiasi cahaya matahari, suhu persekitaran dan hujan terhadap kadar pelesapan racun perosak. Didapati kadar pelesapan bagi ketiga-tiga racun perosak pada sayuran adan tanah di bawah iklim tropika adalah lebih cepat berbanding kawasan beriklim sederhana serta iklim sub-tropika bershu rendah. Residu λ-cyhalothrin menepati tahap piawaian pada hari ketiga manakala profenofos dan chlorothalonil mengambil masa agak panjang (14-22 hari). Tempoh masa menunggu yang lama menunjukkan penggunaan kedua-dua racun perosak ini pada sawi hijau harus dielakkan 7 hari sebelum tempoh penuaian.

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LIST OF ABBREVIATIONS

a.i	Active ingredient
C_i	Initial concentration
CO_2	Carbon dioxide
DT_{50}	Deterioration time
GC-ECD	Gas chromatography with electron captured detector
GC-FPD	Gas chromatography with flame photometric detector
h	Hour
HCN	Hydrogen cyanide
k	Rate constant
K_d	Soil/water adsorption coefficient
K_{ow}	Octanol/water partition coefficient
Koc	Soil Organic Carbon-Water Partitioning Coefficient
MRL	Maximum residue limit
MgSO4	Magnesium sulphate
$mgkg^{-1}$	Milligrams per kilogram
mgL^{-1}	Milligrams per litre
min	Minute
mPa	Millipascal
NaCl	Sodium chloride
N.H	Net house
OC	Organochlorine
OP	Organophosphorus
O.F	Open field
PHI	Preharvest interval
Py	Pyrethroid
PSA	Primary secondary amine
r.h	Relative humidity
t	Time
$t_{1/2}$	Half-life
USEPA	United States Environmental Protection Agency

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CHAPTER 1

INTRODUCTION

1.1 Vegetable production in Sarawak

Vegetable production is a profitable business for local farmers, where demand is increasing every day. As population grow, demand for foods increased steadily, which include demands for fresh vegetables. Increasing health-conscious and knowledge of health benefits from vegetable consumption have led to greater demand for fresh vegetables (Sheng *et al.*, 2009). In Sarawak, vegetables production areas has increased from 3845-4395 hectares from 2007-2011 (DOA Sarawak, 2012). Three main types of vegetables planted are leafy vegetables which include leaf mustard, Chinese cabbage and sweet shoots; non-leafy vegetables such as eggplant and bitter gourd; and legume; such as long bean and okra (DOA Sarawak, 2012).

The leaf mustard (Brassica juncea [L.] Coss.), or commonly known as the green mustard, is the most widely planted leafy green vegetable in Sarawak with the planting areas of 305 hectares, giving a production output of 3351 metric tonnes in 2012 (Figure 1.0). Other commonly planted vegetables are *kangkong* (water spinach), Chinese mustard, *cangkuk manis* (sweet shoots) and Chinese kale. These leafy green vegetables have short crops cycle where they can be harvested within four to six weeks after planting (Talekar *et al.*, 2003).

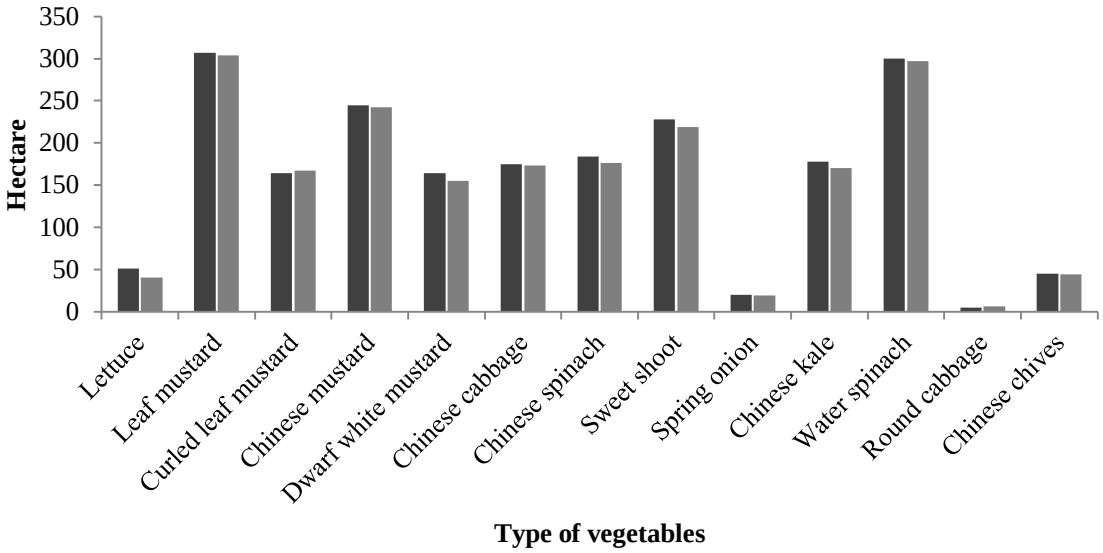


Figure 1.0 Estimated area of leafy vegetables planted in Sarawak for the year 2011 (■) and 2012 (■) (DOA Sarawak, 2012).

Leafy green vegetables are perishable and have short shelf-life. Therefore they must be sold within a few hours after harvested. Demands for fresh looking vegetables, with no apparent bruises, discoloration and holes attributed to pest infestation and plant diseases have put pressure on farmers to produce good quality vegetables. Besides, good quality vegetables are more preferable and fetch higher price in the market. A constrain to produce high quality vegetables in a large quantity leading to overuse of agrochemicals such as pesticides and fertilizers (PAN Asia Pasific, 2010; Fantke & Juraske, 2013).

1.2 Cultivation systems

Two types of cultivation systems for vegetable cultivation commonly used by the local farmers are the net houses (N.H) and open field (O.F). Traditionally, all vegetables are grown in O.F. Pests attack in field is controlled with pesticides. N.H cultivation systems were later introduced to reduce excessive solar radiation and other damaging weather effects on crops, keeping away insect pests and hence producing pesticides free vegetables

(Talekar *et al.,* 2003; Majumdar, 2012). Reduction of sunlight radiation due to shading and temperature build up due to its hermetic condition is common inside N.H (Fang *et al.,* 2006).

Although N.H was invented to provide protection from pests attack, damage due to pest infestation is still common as some insects are able to penetrate the netting mesh and some pests and plant diseases are soil-borne (Talekar *et al.,* 2003). The warm, humid, wind-free and hermetic conditions in N.H may cause more severe crops damage compare to O.F (Huang & Jarvis, 2002). Therefore, farmers still using pesticides in N.H to protect their vegetables from damage by pests and diseases.

1.3 Usage of pesticides in vegetable cultivation

Continuous and intensive cropping often led to excessive build-up of pests and diseases in vegetable production areas (Huang & Jarvis, 2002). Besides, the local climate which is warm and humid favours the resurgence of pests and plant diseases (Niir Board, 2004; Chai *et al.,* 2008). These problems have led to the high usage of pesticides in vegetables production. As developed countries have phased out the usage of toxic pesticides, developing countries are still heavily relying on these pesticides leading to more risk of toxic pesticides residue exposure (PAN Asia Pasific, 2010).

Farmers in developing countries have less awareness and knowledge on the safe usage and possible health risk caused by pesticides (Dinham, 2003). Unregistered and even banned and restricted pesticides are still widely used as they are cheaper and more effective (Waichman *et al.,* 2007). Without proper knowledge, some farmers used mixture of pesticides and increased applied dosage believing it would be more effective in controlling

pests (PAN Asia Pasific, 2010). Injudicious use and violation of pesticides in vegetable productions had led to serious residual problems in local vegetables produced. This could potentially bring health hazard to the consumers as most vegetables are taken raw and undercooked (Juraske *et al.,* 2007).

1.3.1 Pesticide impact on food safety

Although pesticides are credited with success in increasing farm yield and ensuring good quality crops, their potential to harm human has received greater attentions and became an important issue for discussion over the past few decades (Juraske *et al.,* 2007). Pesticides are considered as a significant source of diffuse pollutants that can cause health implications upon human (Juraske *et al.,* 2009). Human generally exposed to pesticide *via* consumption of agricultural commodities and drinking water containing pesticide residue (Fantke & Juraske, 2013). Intake of pesticides residue through foods has been shown to be up to 5 orders of magnitude higher than other exposure routes (Juraske *et al.,* 2007).

In developed countries, the potential lifelong damage of pesticides to human health due to consumption of vegetable and fruits was estimated to be 4.2 minutes of life lost for each individual (Juraske *et al.,* 2009). The potential risk of lifelong damage may be greater in developing countries where food control programmes have not properly established, banned pesticide products are still widely used and farmers are not well trained in relation to good agriculture practice, GAP (Juraske *et al.,* 2009; PAN Asia Pasific, 2010). Although the estimated potential damage of pesticide seems to be small, the impact of pesticides would be greater for infants, children, diabetic and pregnant woman whom are more sensitive to pesticides (Juraske *et al.,* 2009).

Food safety is monitor accordingly to protect the public against health risk due to consumption of food containing unwanted substances such as pesticide residue (Codex Alimentarius Commission, 2013). Intake of pesticide residue in human can be estimated by calculating the ADI (acceptable dietary intake) of the consumer (Darko & Akoto, 2008). ADI is an estimated amount of a substance in food (expressed on a body-weight basis) that can be ingested daily over a lifetime without appreciable health risks to the consumer (Darko & Akoto, 2008). The estimated ADI should be lower than established ADI of the World Health Organisation, WHO (Darko & Akoto, 2008).

The amount of pesticide residue in agriculture commodities was restricted to a certain level established by authorised body in order to protect the consumers from risk of food toxicity. Vegetables and any other commodities produced must not contain pesticide residue above MRL as stipulated in the Food Regulation 1985 (Legal Research Board, 2012). MRL refers to the maximum concentration of a pesticide residue (expressed as mg/kg), recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feeds (Codex Alimentarius Commission, 2013). It serves as a reference on the production, trade of agricultural commodities, and the control and inspection of commodities on sale, import and export (Codex Alimentarius Commission, 2013).

MRL values derived from repeated field trials, where the crop has been treated according to GAP and an appropriate pre harvest interval (PHI) or withholding period has elapsed (Hamilton, 2002; Codex Alimentarius Commission, 2013). The presence of pesticide residue which exceeded MRL will constitute exporting barriers to local produce and could pose significant health risk to the consumers if it presence in a high concentration or after a prolong intake (Juraske *et al.*, 2007).

In Sarawak, the enforcement of food safety is carried out by regular inspection of food products, which includes fresh agriculture commodities. Food is monitored continuously to protect the public against health hazard and fraud in foods production. This step is imperative to know the pesticide residue level in commodities produced in order to protect the consumers and to ensure food safety. Some pesticides are frequently found in vegetable samples analysed with residue above the MRL are shown in Table 1.0. The pesticides were mostly belongs to the organophosphorus (OP) and synthetic pyrethroid (Py) groups indicating the farmers are shifting from the toxic and persistent organochlorine (OC) to the degradable groups of pesticides.

Table 1.0 Types of pesticide with residue exceed MRL found in vegetable samples analysed in 2011 (DOA, 2012)

Pesticide	Total samples exceeded MRL	Residue levels detected (mg kg^{-1})
Profenofos	28	0.02-0.82
Chlorothalonil	17	0.02-0.32
λ-cyhalothrin	8	0.01-0.08
Deltamethrin	4	0.04-0.56
Dimethoate	13	0.02-1.48
Malathion	2	0.48-2.50

According to Table 1.0, profenofos was frequently found to exceed MRL in vegetable samples analysed with residue level of 0.02-0.82 mg kg^{-1}. The highest residue ever detected in 2011 was malathion (0.48-2.50 mg kg^{-1}) followed by dimethoate (0.02-1.48 mg kg^{-1}). These three pesticides (profenofos, malathion and dimethoate) belong to the OP group. Chlorothalonil was also found to exceed MRL regularly in vegetables analysed with 17 samples found to contain chlorothalonil residue above the permissible limit. Chlorothalonil is a fungicide, usually applied onto crops and soil to control plant disease

vectors (Cox, 1997). Λ-cyhalothrin and deltamethrin (Py group) residues were also frequently found above MRL. The Py pesticides is widely used by local farmers as it gained popularity due to its shorter PHI and highly effective.

Though OP and Py pesticides are reported to be easily degraded, their residues found in commodities produced indicated considerable persistency due to some possible causes. This may be due to early harvest (PHI not obeyed), improper usage of pesticide (where pesticide meant to be used in cash crops was used on food crops), or extensive application irrespective of the dosage prescribed (Waichman *et al.*, 2007). If the farmers follow directions on the label properly and practice GAP, violation cases would be unlikely to happen. The dissipation rate of pesticides depends on local environmental condition. Thus, extrapolated data on a pesticides label may turn out to be different as MRL and pre-harvest intervals are by their nature varies depending on local condition (Hamilton, 2002).

1.3.2 Pesticide impact on the environment

Pesticide application in vegetable production has led to the disproportionate introduction of pesticides into soil via spray drift; wash-off from plant and soil drenching (Ciglash *et al.*, 2006; Chai *et al.*, 2008). This has caused environmental problems such as the contamination of water resources and alteration of soil natural properties (Sposito, 2008). Persistent and highly soluble pesticides are susceptible to leaching, thus it has greater potential to contaminate ground water (Ngan *et al.*, 2005). Insoluble pesticides may pose no threat to the ground water as they are tightly bound to soil particles and immobile (Picó & Andreu, 2004). However, preferential flows would enable insoluble pesticide to reach the ground water (Laabs *et al.*, 2002). Besides, they may also pose a problem to surface

water if the contaminated soil eroded from the land and carried into nearby water bodies (Chai *et al.*, 2008).

These have led to considerable research on the environmental fate of a pesticide as it come into contact with soil and enters the environment. Besides, a comprehensive understanding of its fate and movement under actual specific environmental and climatic condition is necessary in order to develop appropriate management strategies of pesticide in agricultural sectors and in the assessment of their potential detrimental effects to the environment (Racke *et al.*, 1997).

Therefore, in order to conciliate agricultural and environmental interests, a better understanding of the fate of pesticides in both environment and in plant is needed. As the behaviour of each pesticide differs greatly upon their physico-chemical properties and types of formulation and they may also behave differently under different climatic and environmental conditions, the assessment should be done individually (Hamilton, 2002; Fenoll *et al.*, 2009). Field study conducted under the local condition may reveal different pattern of pesticides behaviour due to variation in climate condition. Therefore in order to understand how a pesticide behave in both crops and soil, an experiment need to be done following the real practice of farmers.

By conducting field and laboratory studies of a pesticide, the fate and dissipation rate of the individual pesticide after application onto crops and soil, the residual behaviour in the environment and treated crops in terms of half-life and also the optimum pre-harvest interval (PHI) can be determined (Racke *et al.*, 1997; Laabs *et al.*, 2002; Fenoll *et al.*, 2009).

1.4 Objectives

The objectives of this study are;

i. To determine the dissipation rate, half-life ($t_{1/2}$) and pre-harvest intervals (PHI) of profenofos, λ-cyhalothrin and chlorothalonil in green mustard and soil under net house and open field condition.

ii. To evaluate the dissipation dynamic of profenofos, λ-cyhalothrin and chlorothalonil in green mustard and soil.

iii. To determine the suitability of these three pesticides to be used on leafy vegetables with short crop cycle such as green mustard.

iv. To evaluate the degradation of profenofos, λ-cyhalothrin and chlorothalonil in Semongok mineral soil under field and laboratory conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides

Pesticide refers to any substance intended for controlling, preventing, destroying, attracting, and repelling any unwanted species of organisms (plant, fungi, animals) that may harm human and animals, detrimental to plant life and interfering the food chains (Codex Alimentarius Commission, 2001). Pesticides are usually used either before or after harvest in order to protect the commodity from deterioration during storage, transport, distribution and processing of food, agricultural commodities, or animal feeds (Stephenson *et al.*, 2006).

2.2 Types of pesticide

There are two types of pesticide, contact or non-systemic pesticide and systemic pesticide. Pesticides can be classified further into several classes with respect to their functions. There are insecticides (used to control insect pests), fungicides (used to control fungal infection in plant), herbicides (used to control weeds), nematicides (used to control nematodes) and rodenticides (to control rodents). Each class can be divided further into groups of pesticides according to their physicochemical properties (Cremlyn, 1991).

In agricultural sectors, specifically in vegetable production, insecticides and fungicides are amongst the most widely used for crops protection from damage due to plant diseases and infestation of pests. Insecticides can be grouped further into organophosphorus (OP), organochlorine (OC), pyrethroid (Py), carbamate, organic, inorganic, and fumigant

(Cremlyn, 1991). The OP and Py insecticides are the most widely used after the restriction of most OC insecticides because of their high persistency in the environment and are very toxic to animals and human (Smith, 2004).

2.3 The organophosphorus insecticides

OP insecticides form the major and most widely used group that account for more than 36% of the total world market (Porto *et al.,* 2011). OP compounds are degradable organic compounds used primarily in pest control as an alternative to OC compounds that persist in the environment and are more toxic to animals and human (Porto *et al.,* 2011). The OP insecticide consists of phosphoric acid triesters. It has electrophilic phosphorus atom which enable this compound to phosphorylate any nucleophiles including biological nucleophiles (Robert & Hutson, 1985). This ability is supported by the property of 4-nitrophenyl substituent as a good leaving group in the SN_2 phosphorylation reaction. This reaction is the central mode of action of OP insecticides (Robert & Hutson, 1985).

OP insecticides possess an efficient insecticidal activity where it irreversibly inhibits the enzyme acetylcholinesterase in the nervous system of both insects and in mammal (Porto *et al.,* 2011). Although the OP insecticides do not accumulate in human body as it is readily bio-transformed in the liver to water soluble metabolites and excreted in urine, they are still consider to be toxic and may pose some risks to human health (Porto *et al.,* 2011). The OP insecticides were postulated to have the possible ability to methylate DNA and cause damage to the mammalian gene leading to mutation and/or carcinogenesis (Robert & Hutson, 1985).

Some examples of the OP insecticides are profenofos, parathion-methyl, chlorpyriphos, malathion, monochrotophos, diazinon, fenitrothion, and dimethoate. OP insecticides are used to control a variety of sucking, chewing and boring insects, spider mites, aphids, and pests that attack crops like cotton, sugarcane, peanuts, tobacco, vegetables, fruits and ornamentals (Porto *et al.*, 2011).

2.3.1 Profenofos

Profenofos (o-4-bromo-2-chlorophenyl o-ethyl s-propyl phosphorothioate), is a biodegradable wide-spectrum OP insecticide used to control caterpillars and lepidopteron pests of cotton, maize, vegetables, and tobacco (Figure 2.1). Profenofos appears as a light brown liquid with strong smell of cooked onion. Profenofos is hydrophilic with water solubility of 28 mg L^{-1} at 25°C under pH 6.9 and vapour pressure of profenofos is $1.24x10^{-1}$ mPa at 25°C (Worthing & Hance, 1991).

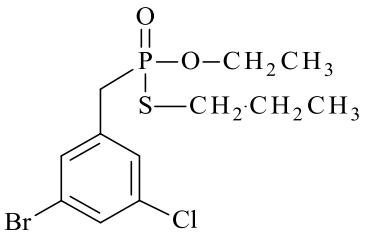
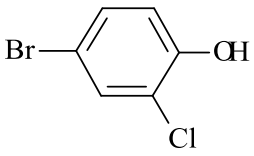


Figure 2.1 Chemical structure of profenofos (Worthing & Hance, 1991)

Profenofos is stable under neutral and slightly acidic conditions but it is hydrolysed in alkaline solution with median dissipation time (DT50) value of 93 days, 14.6 days and 5.7 hours in pH 5, 7 and 9 respectively (Robert & Hutson, 1999). Profenofos has been classified as a moderately hazardous (toxicity class II) pesticide by the World Health Organisation (WHO) where it has moderate order of acute toxicity following oral ingestion

and dermal exposure route. It has high mammalian toxicity and is also very toxic to fish and micro-invertebrates (Worthing & Hance, 1991).

Following of its wide application in agricultural sectors especially in cotton and tobacco plantation, the fate and behaviour of profenofos in plant and soil has been reported (Ismail *et al*., 1993; Sanson, 1994; Wan Abdullah, 1999; Sanmeir, 2003; Ngan *et al.*, 2005). Besides, profenofos residues in other edible crops such as potatoes (Habiba *et al.*, 1992; El-Tantawy *et al.*, 1992), tomatoes (Ramadan, 1991; El-Nabarawy *et al.*, 1992; Ismail *et al.*, 1993), hot pepper, sweet pepper, eggplant (Radwan *et al.*, 2005), and bitter-gourd (Gupta *et al.*, 2012) have also been reported. In plant, profenofos main degradation pathway is *via* hydrolysis, to form compound 1 as in Figure 2.2. Compound 1 was reported to be the main metabolite of profenofos in plant (Irie, 2008). Besides, direct wash-off by rains may contribute to the dissipation of profenofos from plant surfaces due to its high water solubility (Ngan *et al*., 2005).



Compound 1

Figure 2.2 Metabolite of profenofos after degradation (Irie, 2008)

Profenofos was reported to have short half-lives in plant ranging from 1 day up to 5.4 days. The half-life of profenofos in plant depend on several factors such as the type of plant, experimental location climate condition (sunshine, rainfall, relative humidity, temperature), application rate and formulation of pesticides used (Radwan *et al.*, 2004; Nath *et al.*, 2005; Gupta *et al.*, 2011; Gupta *et al.*, 2012). A field study has been carried out by Sanson (1994) on profenofos fate in cotton. It was contended that profenofos was metabolised to

compound 1 which conjugated further to sugars and be likely incorporated into structural components of the plant cell wall (Sanson, 1994). Another field experiment findings reported by Sanmeier (2003), where the metabolism of profenofos in field grown tomatoes were studied showed that profenofos was also degraded into compound 1 via chemical hydrolysis and enzymatic cleavage. Other water soluble complex conjugates consisting of various sugar moieties of compound 1 were also found to be present in the extract (Sanmeier, 2003).

In soil, profenofos was reported to dissipate fast within the period of 15 days (Wan Abdullah, 1999). The dissipation rate depend greatly on factors such as type of soil, pesticide's formulation used, applied dosage, and local geographical and climatic condition (Ngan *et al.,* 2005). Under field condition, profenofos dissipation from soil occurred mainly via leaching and biodegradation (Ngan *et al.,* 2005; Malghani *et al.,* 2009). Profenofos has high water solubility, thus it can easily leached through soil profile (Ngan *et al.,* 2005). However, its residue was not found in runoff water because although it has high water solubility, profenofos has a short half-life of 8 days under field condition (Wan Abdullah, 1999; Ngan *et al.,* 2005).

On the other hand, Das (1991) has investigated metabolism of profenofos in sterile and non-sterile soil under controlled aerobic and anaerobic condition. Metabolism of profenofos in soil occurs via mineralisation activity of the soil microbes. This metabolism activity led to the oxidation of phenyl ring in profenofos (Das, 1991). Mineralisation activities were measured by the amount of carbon dioxide evolved throughout the incubation period. Das and associates carried out incubation study using autoclaved and fresh soil. No significant evolution of CO_2 occurred throughout the experimental period

and no polar compound formed in sterile soil suggested microbial involvement in the formation of polar compounds in non-sterile soil. Mineralisation of profenofos in non-sterile soil occurred actively under aerobic condition resulted to average deterioration time (DT50) of 1.9 days (Das, 1991).

2.4 The pyrethroid insecticides

Pyrethroid (Py) is a new group of insecticides which has gain popularity because of their effectiveness and broad spectrum insecticidal activity. They are extensively used and represent the second largest of the most widely used insecticides. Py insecticides are pyrethrin's analogue (Shukla & Omkar, 1998). Pyrethrin is the active insecticidal constituent present in the pyrethrum (powdered dried flower of *Chrysanthemum cinerariaefolium*). Pyrethrin has good insecticidal properties but degraded rapidly in the presence of air and sunlight, thus limiting their application to household and indoor use (Shukla & Omkar, 1998). Py insecticides were synthesized with slight structural modifications in the acid as well as the alcohol moieties of pyrethrin to yield photostable analogues of Py with half-life of several days (Shukla & Omkar, 1998).

Allethrin was the first pyrethrin analogue synthesized and was the first commercial Py insecticide introduced as a mixture of eight isomers. However, they are still unstable to air and sunlight. The effort of developing photostable Py with higher insecticidal activity resulted in the development of permethrin, cypermethrin and deltamethrin which are more stable in air and light, and more toxic to insects (Shukla & Omkar, 1998).

Development of Py insecticides with stability better than organophosphates, effectiveness against wide range of insects and more favourable environmental impact make them the

compound of choice for agriculture (Shukla & Omkar, 1998). Besides, they are less hazardous towards honey bees compared to OP insecticides which are highly toxic to honey bees (Cremlyn, 1991). Although Py insecticides may also persist on crops surface, they do not migrate and are biodegradable. This has led to extensive introduction of Py insecticides such as cyhalothrin, cyfluthrin and fenvelerate in agricultural sector (Shukla & Omkar, 1998).

Py insecticides have poor water solubility, therefore they are highly lipophilic. They are prone to be absorbed by waxy layers of plant to kill and repel insects that infest the plant. Although Py insecticides have low toxicity towards mammals, they are highly toxic to freshwater and marine organisms (fish, crustaceans and aquatic insects) (Shukla & Omkar, 1998). Due to their hydrophobicity, Py insecticides may form strong bonding with soil particle. Therefore, they are immobile in soil and thus leaching through soil into the groundwater is very unlikely (Demoute, 1989). However, translocation via soil erosion and run-off may cause contamination of nearby surface water (Chai *et al.*, 2009). Besides, preferential flow through soil macropores may enable bound residue of Py insecticides to reach ground water (Laabs *et al.*, 2002).

The persistency of Py insecticides residue in soil varies considerably, where some are relatively stable and some are photosensitive. Photosensitive Py insecticides have shorter half-life, while photostable Py insecticides have much longer half-life in the environment. Some Py insecticides are biodegraded by soil microbes leading to carbon dioxide (CO_2) formation, while some may persist longer in soil (Demoute, 1989).

In agriculture, Py insecticides are commonly used to control aphid, coleopterous, and lepidopterous pests in targeted crops include cotton, cereals, hops, ornamentals, potatoes,

and vegetables (Cremlyn, 1991). Py insecticides are also used in public health management where applications are made to control cockroaches, mosquitoes, ticks, and flies, which may act as disease vectors (Cremlyn, 1991).

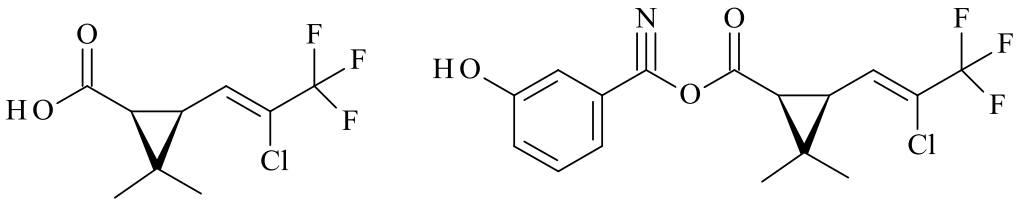
2.4.1 λ-cyhalothrin

λ-cyhalothrin is a 1:1 mixture of (S)-α-cyano-3-phenoxybenzyl-(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclopropane carboxylate and (R)-α-cyano- 3 phenoxybenzyl-(Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethylcyclo - propane carboxylate. At room temperature, λ-cyhalothrin appears as a colourless solid at room temperature but may appear yellowish in solution. It has a low vapour pressure (2.00^{-7} mPa at 20 °C) and a low Henry's law constant, which suggests that it is not easily volatilized into the atmosphere (Tomlin, 2000).

λ-cyhalothrin also has a high octanol–water partition coefficient (log K_{ow}) which is 7.00. It has low solubility in water (0.004 mg L^{-1} at 20 °C) and tends to partition into lipids, which leads to its high potential to bio-concentrate (He *et al.,* 2008). The high water–soil organic carbon partition coefficient (K_{oc}) value indicates its preferential affinity to organic matter. This would suggest that it has low potential to leach as dissolved residue in percolating water (He *et al.,* 2008). λ-cyhalothrin is quite stable in water at pH < 8, whereas under alkaline conditions it hydrolyses through nucleophilic attack of the hydroxyl ion forming a cyanohydrin derivative which degrades to form hydrogen cyanide (HCN) and aldehyde under alkaline conditions (He *et al.,* 2008).

λ-cyhalothrin works by penetrating the insect's cuticle and disrupting nerve conduction within minutes. This leads to cessation of feeding, loss of muscular control, paralysis, and

eventual death (He *et al.*, 2008). Additional protection of the crop is provided by its strong repellent effect toward insects. In plant, the metabolism of λ-cyhalothrin is limited to a few transformation steps. The cleavage of the ester bond is normally the first step of λ-cyhalothrin breakdown, forming 2 and 3 (Figure 2.3) followed by hydroxylation of the breakdown products into CO_2 and water (Fernandez-Alvarez *et al.*, 2007; He *et al.*, 2008).



2 3

Figure 2.3 Plausible λ-cyhalothrin metabolites formed in plant and soil (He *et al.*, 2008).

λ-cyhalothrin fate in soil has been studied (Robert & Hutson, 1999; Tariq *et al.*, 2006). It was suggested that λ-cyhalothrin has low potential to leach as dissolve residue because of its high water–soil organic carbon partition coefficient (K_{oc}) value and low solubility in water. Therefore, λ-cyhalothrin is usually found in the top 0-10 cm layer of soil. However, it was reported that the residue was also detected in the deeper 10–30 cm soil layer suspected to result from preferential flow (Tariq *et al.*, 2006).

λ-cyhalothrin degraded in soil *via* several pathways depending on the soil condition. For example, under anaerobic condition, λ-cyhalothrin degraded mainly via hydrolysis forming the acids while under aerobic condition, it degraded *via* mineralisation where ester-bond cleavage and hydroxylation took place forming 4-hydroxy-cyhalothrin followed by a rapid generation of $^{14}CO_2$ (Robert & Hutson, 1999). Hill and Inaba (1991) have pointed out that the degradation pathway of Py including λ-cyhalothrin in soil is mostly microbial. The degradation rate was much faster in cropped soil compare to fallow soil. This is due to the

optimum moisture and temperature level for microbial growth and activity in soil under crop canopy (Hill & Inaba, 1991).

2.5 The fungicide

Fungicides are either chemicals or biological agents that protect plants from fungal diseases by inhibiting the growth of fungi or fungal spores (Hewitt, 1998). Plant infested by fungi can cause serious damage in agriculture, resulting in critical losses of crops yield, quality and profit. Fungicides are used both in agriculture and to fight fungal infections in animals. Fungicides can be classified into two classes according to their mode of action which are protectant or surface fungicides and also systemic or chemotherapeutants fungicides (Cremlyn, 1991).

Protectant or surface fungicides are usually applied to plant foliage as dusts or spray. They do not penetrate the plant cuticle, but they only protect the plant surface where the spray is deposited. On the other hand, systemic fungicides translocate within the plant following application onto soil. They are absorbed *via* roots or seeds and are distributed through the xylem vessels to the upper part or the other part of the plant (Cremlyn, 1991).

Majority of fungicides are not very stable in the environment. Their stability in soil depends greatly upon the chemical structure, nature of the soil, and general climatic conditions (Cremlyn, 1991). The earliest fungicides introduced were inorganic materials such as sulphur, copper and mercury compounds. Later, fungicides introduced were heavy metal derivatives, dithiocarbamates, quinones, phtalimides and chlorothalonil (Cremlyn, 1991).

2.5.1 Chlorothalonil

Chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile), is a foliar, non-systemic and broad-spectrum chlorinated fungicide. It is widely applied for the control of a variety of fungal diseases and pathogens infecting vegetables, fruits, turf and ornamentals in different commodities (Tomlin, 2000). Chlorothalonil appear as white crystalline solid or powder that has a slightly musty odour. Chlorothalonil is soluble in water with solubility of 0.81 mg L^{-1} at 25 °C. It has vapour pressure of 7.62×10^{-5} mPa at 25 °C (Tomlin, 2000).

Chlorothalonil is classified as a 'probable human carcinogen' by the US EPA due to the presence of carcinogenic hexachlorobenzene (Cox, 1997). Chlorothalonil is extremely toxic to fish in acute lethal doses although it undergoes rapid metabolism *via* the glutathione pathway to give water soluble metabolites (Worthing & Hance, 1999; Tomlin, 2000). Chlorothalonil fate and behaviour in plant has been extensively studied (Zhang *et al.,* 2007; Chaves *et al.,* 2007; Chaves *et al.,* 2008). Chlorothalonil dissipation from plant occurred *via* photodegradation, volatilisation; plant uptake and washed-off by rainfall (Zhang *et al.,* 2007; Putnam *et al.,* 2003; Leistra & Van Den Berg, 2007; Monadjemi *et al.,* 2011). Chlorothalonil showed rapid dissipation trend from plant surfaces giving half-life of 1 to 3 days respectively, depending on crop types and environmental conditions. Apart of its rapid dissipation in plant, formation of chlorothalonil bound residue in tomato and apple fruit cuticles was also reported (Potter *et al.,* 2001).

The influence of temperature and sunlight radiation to fungicides dissipation from plant has been studied (Mariän *et al.,* 2003; Fenoll *et al.,* 2009). The dissipation rate of chlorothalonil was much higher in field at ambient temperature with natural light compared to cold conditions and darkness. Chlorothalonil half-lives under refrigeration were reported

to be 5-9 times longer than in the field because the process of evaporation and photodegradation under cold conditions and darkness are negligible (Fenoll *et al.*, 2009). This showed that the role of sunlight radiation in pesticides degradation is remarkably significant as it triggers and initiated chemical reactions, such as oxidation, reduction, catalytic hydrolysation and bond-cleavage (Fantke & Juraske, 2013).

Photodegradation of chlorothalonil on crops mainly occurred via reductive dechlorination (Monadjemi *et al.*, 2011). Extrapolated field dissipation half-life of photolysis on vegetation was estimated to be 5.3 days and chlorothalonil dissipation from crops is ruled by both photodegradation and penetration (Monadjemi *et al.*, 2011). Following foliar application on cranberry in a bog, chlorothalonil followed first-order kinetics with estimated half-life of 12.7 days (Putnam *et al., 2003*).

Volatilisation of chlorothalonil from crop was reported to be a continuous process which contributed to its dissipation from plant (Leistra & Van Den Berg, 2007). Competing processes such as photodegradation and plant penetration were found to significantly affect chlorothalonil volatilisation rate. However, as these competing processes also occurred at a lower rate, chlorothalonil volatilisation continues for a longer time (Leistra & Van Den Berg, 2007).

Chlorothalonil is susceptible towards rainfall wash-off. Rainfall has been reported to show greater removal effect of fungicide deposits on plant compared to the other climatic factors such as sunlight and temperature (Bruhn & Fry, 1982; Fife & Nokes, 2002). The effect of rainfall on chlorothalonil removal has been found to have greatest impact if rainfall occurs on the day of application or soon after, and the effect of rain declines with times (Bruhn

and Fry, 1982). A small amount of rain was reported to be sufficient in removing a large amount of the original chlorothalonil deposit on plant. However, the trace level concentration of chlorothalonil appeared to be more tenacity than the higher concentration (Bruhn and Fry, 1982; Fife & Nokes, 2002). After readily dislodgeable chlorothalonil is removed from the foliage by rain, it appears that additional rain does not remove the remaining chlorothalonil residue (Xu *et al.*, 2008). The remaining chlorothalonil residue which presence in a very low concentration was suspected to be held within the leaf matrix, therefore it is not easily removed by additional rain (Fife & Nokes, 2002).

The effect of rainfall on chlorothalonil dissipation from plant was also found to be varied according to rainfall intensity, rainfall duration and rainfall frequency (Fife & Nokes, 2002). Fife and Nokes, (2002) pointed out that mean chlorothalonil residues loss for plants exposed to a rainfall intensity of 25 mm h^{-1} were higher compared to plants exposed to intensity levels >13 mm h^{-1}, provided the rainfall duration period was short. However, for longer rainfall duration, the influence of rainfall duration period towards foliar chlorothalonil loss became more pronounced, and rainfall intensity was no longer significant (Fife & Nokes, 2002).

Several studies have been carried out to investigate the degradation of chlorothalonil in soil (Rouchaud *et al.*, 1988; Motonaga *et al.*, 2002; Regitano *et al.*, 2001; Chen *et al.*, 2001; Putnam et al., 2003; Ngan *et al.*, 2005; Chaves *et al.*, 2007). Main dissipation pathways of chlorothalonil in the environment are dissipation *via* volatilisation, photodegradation, biodegradation, sorption to soil and humic substance (Katayama *et al.*, 1995; Regitano *et al.*, 2001). Compound 4 (Figure 2.4) has been reported to be the most predominant metabolite of chlorothalonil found in soil (Rouchaud *et al.*, 1988; Ukai *et al.*, 2003). This

metabolite was reported to be more persistent, mobile, and toxic than the parent compound, resulting in suppression of soil microorganisms (Chen *et al.*, 2001; Ukai *et al.*, 2003).

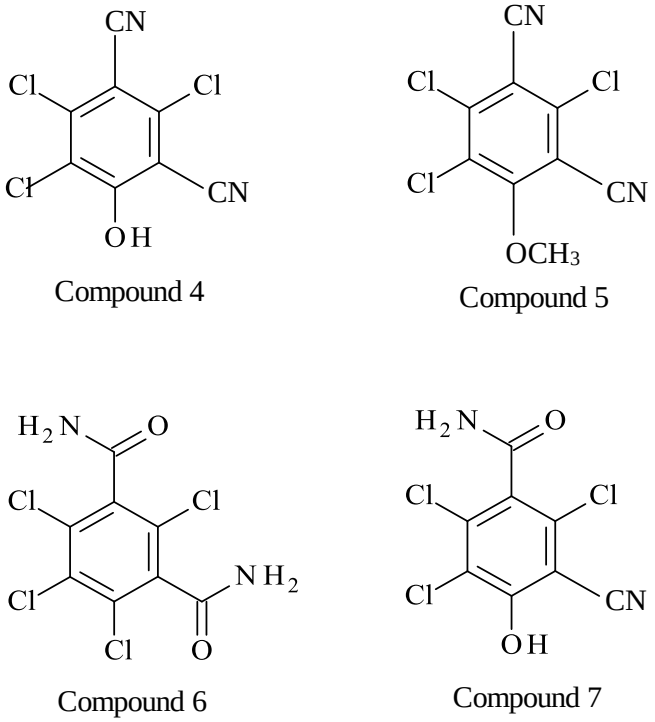


Figure 2.4 Plausible chlorothalonil metabolites in soil (Chaves *et al.*, 2008)

Chen *et al.*, (2001) investigated the extent of the influence of fungicides on soil microorganisms and microbial processes in soil. Chlorothalonil was found to decrease soil mineralisation rates significantly over the 2 weeks in all three soils tested (Chen *et al.*, 2001). However, after 14 days, soil mineralisation rate increased until the end of the experiment. This phenomenon is due to the recovery of some resistant soil microbial species which directly utilizes chlorothalonil as a carbon sources (Chen *et al.*, 2001). This investigation affirmed the short-term side effects of chlorothalonil on soil microbial activities in natural soil and soil amended with organic compound under laboratory condition.

Other metabolites of chlorothalonil (compound 5–7) reported were the results of reactions such as sulphate reduction and dechlorination leading to the substitution of chlorine (Cl) atoms in chlorothalonil and conversion of the CN functional groups to amides, thiazoles and acidic groups (Rouchaud *et al.,* 1988; Regitano *et al.,* 2001; Chaves *et al*., 2008). Under aerobic condition, chlorothalonil metabolites may degrade further via reductive dechlorination where the halogenated benzene structure will destroy (Carlos-Rojas *et al.,* 2004). Hydrogenation was reported to be not likely to happen in chlorothalonil degradation (Carlos-Rojas *et al.,* 2004).

Tropical soils are usually more acidic than temperate soils and this factor may affect the metabolic pathways of chlorothalonil. Regitano *et al.,* (2001) conducted a series of experiments on the degradation of chlorothalonil involving the incubation of three acidic Brazilian soils samples 90 days. The study was carried out by using the radiolabelled ^{14}C-chlorothalonil technique. Their results showed that although most of the compound was lost within the first 7 days, effective mineralisation was found to occur at a rather slow rate as evaluated by $^{14}CO_2$ assays. Acidic medium was found out to have no effect to chlorothalonil degradation because of its hydrolytic nature of degradation (Rouchaud *et al.,* 1988; Carlos-Rojas *et al.,* 2004).

Chlorothalonil degradation in soil was frequently correlated with abiotic processes. Chlorothalonil degradation occurred in both soil with the presence of microorganisms and without microorganisms (abiotic condition). The difference in the dissipation rate between autoclaved and intact soil was interpreted to be microbial degradation (Katayama *et al.,* 1995). Chlorothalonil showed fast dissipation rate in autoclaved soil amended with farm yard manure compared to original autoclaved soil. Abiotic degradation of chlorothalonil

was then suspected to be due to chemical degradation and sorption to organic matter (Katayama *et al.,* 1995; Regitano *et al.,* 2001).

Another separate study by Regitano and associates affirmed the ability of chlorothalonil to degrade under abiotic condition. Significant fraction of the chlorothalonil that had been assumed to be rapidly degradable in soils has turned out to be soil-bound residues. Most soil-bound ^{14}C residues were formed in the first day, but aging also contributed to the formation of less reversible forms of chlorothalonil-soil complexes (Regitano *et al.,* 2001). Mineralisation therefore turned out to be not a major metabolic pathway of chlorothalonil degradation as reported previously by Katayama and associates.

Chlorothalonil degradation was relatively fast under controlled condition with 88% dissipated during the first 24 hours of incubation (Regitano *et al.,* 2001). Calculated half-lives reported for chlorothalonil under controlled condition ranging from 0.41 to 14 days respectively. While under field condition, half-life of chlorothalonil in soil is not more than 8 days (Ngan *et al.,* 2005; Chaves *et al.,* 2007).

2.6 The dissipation of pesticides from plant and soil

The understanding of pesticide fate and behaviour in plant and soil is important because plant act as the transitional media for pesticide from soil, water and air to enter the food chain, whilst soil act as an entering point to natural water resources and also the food chain (Beiber, 1999). Plants are able to adsorb contaminants from soil *via* root uptake and accumulate pollutants from the atmosphere through deposition on leaf surface followed by stomata and cuticular waxes uptake (Beceiro-González *et al.,* 2012).

Soil on the other hand would lead to the dispersion of pesticides to adjacent ecosystems, leaching to ground water and thus affecting non-target organisms (Ismail *et al.*, 2002; Chai *et al.*, 2014). Pesticide ability to contaminate ground water is governed by its high water solubility, low sorption coefficient and high persistency in the environment. These properties are common in most pesticides produced as pesticide were usually made to be thermolabile and hydrosoluble to facilitate disappearance from the environment and at the same time persist long enough to provide effective pest control (Andreu *et al.*, 2004).

Pesticide introduced to the environment will undergo changes which led to structural alteration, detoxification, deactivation, and finally disappearance of the active ingredients (Sposito, 2008; Malghani *et al.*, 2009). Some diminish quickly and some would persist longer in soil. The degree of pesticides dissipation rely on factors such as pesticide formulation used (wettable powder, oil suspension), physico-chemical property of pesticides (solubility, ionisation, adsorption affinity), climate condition (temperature, sunshine, rainfall, humidity) and plant physiology (Chao *et al.*, 2005; Fan *et al.*, 2006; Fantke, 2013).

2.7 Dissipation mechanisms of pesticide on plant

Following foliar application, pesticide deposited on plant surfaces may undergo dissipation *via* a series of different mechanisms and processes such as chemical degradation, volatilisation, biotransformation, plant growth dilution and wash-off by rainfall (Elliot & Spurr, 1993; Beiber, 1999; Fife & Nokes 2002; Katagi 2004). These processes may occur individually or simultaneously at one time and are governed by many factors such as environmental conditions (relative humidity, rainfall, temperature and UV irradiation), plant morphology (waxy peel, hairy, rough or smooth surface, water and lipid content)

pesticide formulation used, application techniques and dosage applied (Mariän *et al.,* 2003; Ntow *et al.,* 2007; Juraske *et al.,* 2008; Fantke, 2013).

Environmental factors contributed to the variation of pesticide dissipation rate from plant, and thus affecting local pre-harvest intervals and half-life (Hamilton, 2002; Fenoll *et al.,* 2009). A number of dissipation studies have been carried out under different seasons (autumn, spring, winter, and summer) and in different climate such as temperate, tropical, sub-tropical and semi-arid conditions. It is demonstrated that the rate of pesticide disappearance depends on local environmental conditions (Elliott & Spurr, 1993; Fife & Nokes, 2002; Mariän *et al.,* 2003; Fan *et al.,* 2006; Zhang *et al.,* 2007; Chai *et al.,* 2008; Xu *et al.,* 2008; Sapbamrer & Hongsibsong, 2014).

The influence of temperature and sunlight radiation towards pesticide dissipation from plant has been studied (Mariän *et al.,* 2003; Fenoll *et al.,* 2009). The dissipation rate was shown to be much higher in the field at ambient temperature and with the presence of sunlight compare to cold and dark conditions in the cold storage. This was ascribed to the absence of sunlight in the cold storage where evaporation and photodegradation of pesticides are negligible (Fenoll *et al.,* 2009).

2.7.1 Chemical degradation of pesticide in plant

Pesticide deposits on plant surfaces may undergo chemical degradation (Fernandez-Alvarez *et al.,* 2007; He *et al.,* 2008; Fantke *et al.,* 2014). Chemical degradation in/on plant involved numbers of processes including oxidation, reduction, hydrolysis and conjugation reaction (Fantke & Juraske, 2013). These processes can be categorised further into three phases with oxidative, reductive and hydrolisation occur in the initial phase, followed by

conjugative process in the second phase and formation of secondary conjugates and bound residue at the final phase (Fantke & Juraske, 2013).

Photodegradation of pesticides involved chemical reactions, such as oxidation, reduction, catalytic hydrolysation and bond-cleavage which occur with the presence of sunlight radiation (Fernandez-Alvarez *et al.,* 2007; Fantke & Juraske, 2013). The presence of free radicals and photon energy from the sunlight will initiate organic reactions such as decarboxylation or hydroxylation and oxidation of reactive oxygen species of pesticide's functional group (He *et al.,* 2008; Fantke & Juraske, 2013). Studies on pesticides degradation for cabbage, Chinese mustard, Chinese broccoli, lettuce, eggplant, carrot, tomatoes and asparagus demonstrated pesticides dissipation from crops *via* reduction and hydrolysation mechanism in the presence of sunlight radiation (Ripley *et al.,* 2001; Pei *et al.,* 2004; Cao *et al.,* 2005).

2.7.2 Volatilisation of pesticide on plant

Pesticide volatilisation took place on outer layer of plant surfaces. It could be defined as the evaporation of pesticides from plant surfaces as a function of interception areas and roughness, as well as transpiration as a function of inner plant transpiration stream velocity which drove subsequent loss of water as vapour through stomata (Fantke & Juraske, 2013). Volatilisations of pesticides are influenced by their volatility or vapour pressure, temperature and wind velocity or movement (Beiber, 1999).

Pesticides with vapour pressure higher than $1x10^{-3}$ Pa is classified as a highly volatile pesticides, while pesticides with vapour pressure lower than $1x\ 10^{-8}$ Pa is classified to have low volatility (Juraske *et al.,* 2008). Volatilisation of pesticide from plant surfaces usually

occurs during foliage application. It will continue for several hours after the application depending on surrounding temperature, wind velocity and pesticide's formulation. Pesticides that volatilise from leaf surface to air have the tendency to re-deposit onto plant surfaces (Juraske *et al.,* 2008).

2.7.3 Biotrasformation of pesticide in plant

Pesticides that penetrate into plant tissue and up-taken by plant may undergo biotransformation, which occurs *via* plant metabolism and other biotic processes mediated by plants and microorganisms (Juraske *et al.,* 2008). Once a pesticide enters the inner part of plant, they will undergo biological transformation which involves enzyme as catalyst. This enzymatic reaction will modify and transform the structure and toxicological properties of pesticide (Juraske *et al.,* 2008). Plant generally metabolise pesticides to water soluble conjugate compounds and bound residues which are less or non-toxic and can stay in plant (Juraske *et al.,* 2008). Biotransformation is considered to be the route of pesticides detoxification and metabolism in vegetation (Van Erd *et al.,* 2003; Juraske *et al.,* 2008).

2.7.4 Growth dilution of pesticides in plant

Growth dilution factor leads to pesticides dissipation from plant even without physical or chemical dissipation occurred (Metwally *et al.,* 1997). As the plant grows larger the weight and surface area increases. Increase in plant weight and surface area causing dilution of pesticide concentration (Fantke & Juraske, 2013). Observed pesticide concentration decreased with increasing weight of sampled plant even if the pesticide does not degrade at all (Fenoll *et al.,* 2008).

The effect of growth dilution towards pesticides dissipation varies depending to physic-chemical properties of a pesticide and the plant growth rate (Cabras *et al.*, 1996). Growth dilution contributes more to the dissipation of stable pesticides, as other dissipation factors (temperature, rainfall, and sunlight) did not affect stable pesticide's dissipation from plant (Fantke & Juraske, 2013). Plants with higher growth rate results to faster dissipation of pesticides (Metwally *et al.*, 1997). Contributions of growth dilution reported range from about 10% for thifensulfuron-methyl in whole soybean plants and 82% for dimethoate in artichoke head (Fantke & Juraske, 2013)

2.7.5 Wash-off of pesticides from plant

The dissipation of pesticides *via* washing off by rainfall depends on the pesticides solubility, formulation and rainfall intensity (Beiber, 1999; Fife & Nokes, 2002). Water soluble non-systemic pesticides are more susceptible to be washed-off by rainfall. However, for systemic pesticides, rainfall helps the pesticide to penetrate into inner part of plant rather than washing it off (Leistra & Van Den Berg, 2007). Wash-off effect is usually higher if rainfall occurred shortly after pesticide application (Leistra & Van Den Berg, 2007). The wash-off effect decreased in the subsequent days where other dissipation pathways dominate the dissipation of pesticides from plant (Fantke & Juraske, 2013).

Fife and Nokes (2002) highlighted the effect of rainfall intensity, duration and frequency on chlorothalonil dissipation from plant. In a shorter rainfall duration, rainfall with higher intensity showed greater wash-off efficiency compare to lower intensity. However, for longer rainfall duration, rainfall intensity effect towards wash-off efficiency was no longer significant (Fife & Nokes, 2002).

Wash-off effects were also reported to be influenced by the structure of plant surface (Fantke & Juraske, 2013). The effect of plant surface roughness on wash-off effect was provided by the work of Cabras *et al.,* (2001) whom investigated the wash-off effects on grape and orange fruits compared to the leaves. Wash-off effect was found to be higher on grape fruits compared to grape leaves, while the wash-off effect was higher on orange leaves than orange fruits (Cabras *et al.,* 2001).

2.8 The dissipation mechanisms of pesticides in soil

Pesticide enters the soil *via* several routes such as direct application (for soil drenching purposes), washed-off from treated foliar or spray drift during foliar application (Ciglash *et al.,* 2006). The fate of pesticides in soil involved a number of chemical and physical processes. These processes can be grouped into those that affect persistency and those that affect mobility. Degradation process (chemical and microbial degradation) determined pesticide's persistency in the environment whilst sorption, plant uptake, volatilization, wind erosion, run-off and leaching affect mobility of pesticide (Picó & Andreu, 2004).

Each individual process is controlled by soil physicochemical properties such as clay and mineral content, organic matter content, pH, and water content (Roy *et al.,* 2000; Yu & Zhou, 2005; Ciglash *et al.,* 2006; Sposito, 2008). Besides, climate condition, geographical property and the physicochemical property of pesticides may also lead to the variation of pesticide fate and behaviour in soil (Rice *et al.,* 2002; Picó & Andreu, 2004; Laabs *et al.,* 2002).

Natural soil minerals such as the silicate minerals (clay), oxides and hydroxides provide more binding sites for pesticide adsorption in soil. These minerals are usually hydrophilic

due to the presence of hydroxyl groups and exchangeable cation on its surfaces (Yu & Zhou, 2005; Sposito, 2008). Laabs and Amelung (2005) demonstrated higher affinity of clayey Ustox Brazilian soil for pesticides compared to sandy soil. Soil mineral content was also reported to affect non-polar pesticide leaching in soil due to their strong sorption (Laabs *et al.,* 2002). Although pesticide adsorption may significantly retained pesticide longer in soil by reducing their mobility in soil, preferential flow however was deduced to enable vertical movement of strongly sorbed pesticide in soil (Laabs *et al.,* 2002). Via this preferential flow, persistent pesticide which adsorbed into soil particles are able to move into deeper layer of soil through macromolecular passages apart from being transported to adjacent areas via surface runoff and soil erosion (Laabs *et al*., 2002).

Apart from the presence of soil minerals, the presence of soil organic matter has been well correlated with the increasing retention and persistency of some pesticides in soil (Sposito, 2008). Soil organic matters enhance soil adsorption capacity and at the same time reducing pesticide's bioavailability. Two types of soil organic matters are humic and non-humic soil organic matter. Humic soil organic matter contains humus formed from the transformation of plant, microbial and animal litter by microorganism activity (Tan, 2011). On the other hand, the non-humic soil organic matter contains compounds derived from the decomposition of plants and other organisms with definite characteristic such as lignin, carbohydrates, lipids, protein and amino acids (Tan, 2011).

Soil organic matters are heterogeneous composite containing both hydrophilic and hydrophobic groups (Tan, 2011). Besides, they are acidic where their acidity is attributed to the presence of dissociable protons or H^+ ions in aromatic, aliphatic carboxyl and phenolic hydroxyl groups in its supramolecular structure. The presence of dissociable

protons leads to a spectrum of hydrogen bonding formation within its own supramolecular structure and with exogenous organic compounds. Bonding formation adsorbs pesticide to soil particles leading to pesticide retention in soil. In some instance, they also help in pesticides modification which may detoxify or deactivate their activity (Sposito, 2008).

Soil water content or soil moisture is another important factor which govern pesticides dissipation rate in soil (Racke *et al.,* 1997; Frank *et al.,* 2002). Soil water content defines the specific exchange surface between solid and liquid phases. For polar pesticides, high soil water content may enhance dissipation from the topsoil via leaching (Laabs *et al.,* 2002). For non-polar pesticides such as the Py, the adsorption decreases when the soil water content increases because the hydration of the surfaces of adsorbents decreases the accessibility to adsorption sites. However, soil with low moisture content favour access to the hydrophobic regions of humus by generating more hydrophobic surfaces, thereby increasing the sorption of non-polar pesticides (Roy *et al.,* 2000). Water also acts as hydrolytic agent and it governed hydrolytic and photolytic degradation of most pesticides in soil (Racke *et al.,* 1997; Frank *et al.,* 2002).

Soil pH is also of some importance governing degradation and adsorption process of pesticides in soil. Hydrolysis, oxidation-reduction (redox) and ionisation of pesticides in soil depend greatly on pH. Some pesticide such as atrazine degrades faster in acidic soil, whereas slower degradation was observed for some pesticide such as endosulfan (Ghadiri & Rose, 2001; Laabs *et al.,* 2002). Adsorption increases with decreasing soil pH for ionisable pesticides such as glyphosate and sulcotrione where their adsorption will increase (or decrease) with pH depending on their molecular charge (Chaplain *et al.,* 2011). For example, the retention of glyphosate increases when the soil pH decreases because the

number of negative charges of the molecule decreases, allowing the adsorption on negatively charged adsorbents like clay or organic matter (Yu & Zhou, 2005; Chaplain, *et al.*, 2011).

2.8.1 Volatilisation of pesticides from soil

Volatilisation of pesticide refers to the diffusion of pesticides through soil onto the soil surface and the movement of pesticide into and through the atmosphere (Sposito, 2008). Volatilisation usually took place only on the upper layer of soil. It is dependent on the vapour pressure and heat of vaporisation of the chemical, surrounding temperature, the partition coefficient between the atmosphere and the other phase, and the air flow mass which will transport the airborne chemical (Sposito, 2008).

Volatilisation may cause pesticides dispersion and translocation to distant locations. As pesticides volatility depend to their respective vapour pressure, pesticides with higher vapour pressure will volatilise easily compare to pesticides with low vapour pressure. In addition, pesticides losses due to volatilisation increase with increasing temperature. This is supported by the fact of shorter field dissipation half-life of pesticides in the semiarid and subtropical regions compared to the temperate regions with half-lives less than 15 days (Ciglasch *et al.*, 2006).

2.8.2 Leaching of pesticides in soil

Pesticides dissipation from upper layer of soil under field conditions was also correlated with leaching into deeper layer of soil. The leaching of pesticides in soil has always of great concern as it may cause ground water contamination (Laabs *et al.*, 2002; Kookana *et al.*, 2010). Polar pesticides have low affinity for adsorption to soil thus they are more

susceptible towards leaching which has contributed to their fast dissipation from the top soil layer (Laabs *et al.*, 2002; Ngan *et al.*, 2005; Chai *et al.*, 2009).

Precipitation usually enhanced pesticides leaching in soil where frequent rainfall may cause rapid movement of pesticides into deeper layer of soil (Ciglash *et al.*, 2006). Apart from higher precipitation, the presence of soil macropores may also enhanced pesticide leaching through preferential flow. Preferential flow enables less soluble and insoluble pesticide to leach into the deeper layer of soil as it allows leaching regardless of pesticide polarity (Laabs *et al*., 2002; Chai *et al.*, 2009). Besides, particle-facilitated transport in macropores might have also enabled non-polar pesticides to translocate into deeper soil layer (Laabs *et al.*, 2002). This may prolong their persistency in soil due to lower microbial activities in the subsoil and no losses due to photolysis (Kookana *et al.*, 2010).

2.8.3 Adsorption of pesticides in soil

Adsorption of pesticide to soil particles leads to the retention of pesticide in soil. The retention may control their bioavailability, leaching potential, degradation, and also volatilisation (Yu & Zhou, 2005; Laabs *et al.*, 2005; Sposito, 2008). Adsorption of pesticides to soil particles involve mechanisms such as *Van der waals* attraction, cationic and anionic bonding, hydrogen bonding, charge transfer, ligand exchange, direct and induced ion dipole, dipole-dipole interaction and chemisorption (Sposito, 2008). These mechanisms are controlled by the natural properties and components of soil such as clay and mineral contents, the presence of organic matter, soil pH, and soil water content (Yu & Zhou, 2005; Sposito, 2008). Besides, environmental condition and the physicochemical properties of pesticides also play an important role in pesticides sorption to soil particles.

Pesticides with high affinity for soil have rapid and strong adsorption to soils (Laabs *et al.,* 2005; He *et al.,* 2008). Pesticide affinity is indicated by their soil sorption coefficient (K_d) values determined in a batch equilibrium experiments. Pesticide K_d value is also used for the estimation of pesticide leaching potential (Laabs *et al.,* 2005). Non-polar pesticide demonstrated a fast sorption and attained sorption equilibrium at ≤ 12 h, while more polar pesticides reached an apparent equilibrium only after 24-48 h in topsoil (Laabs *et al.,* 2005).

2.8.4 Degradation of pesticides in soil

Among all dissipation processes, degradation is the most critical part as it may alter and modify the parent compound forming either non-toxic metabolite into simple non-toxic compounds such as CO_2 and H_2O or it may leads to the formation of a more toxic and persistent compounds, which are ultimately undesirable. Degradation of pesticides in soil may occur via photodegradation, chemical degradation (hydrolysis, ionisation, oxidation and reduction) and biological degradation pathway (Chai *et al.,* 2010; Frank *et al.,* 2002; Ghadiri & Rose, 2001). Each degradation pathway may produce a number of different metabolites and end products for a single pesticide (Frank *et al.,* 2002; He *et al.,* 2008).

2.8.4.1 Photodegradation of pesticide in soil

Photodegradation is a chemical degradation of pesticides with the presence of reactive species initiated by sunlight radiation (Frank *et al.,* 2002; Fernandez-Alvarez *et al.,* 2007). Photodegradation occurred *via* chemical bond breakdown as sunlight imparts photolytic energy upon the chemicals. Soil exposed to sunlight leads to the formation of various reactive ions such as singlet oxygen and hydroxyl radical (with the presence of water) at the soil surface (Frank *et al.,* 2002). Photolysis were found to be effective only in the upper

layer (< 2.0 mm depth) where the amount of extractable experimental material decreased with increasing in soil depth (Frank *et al.*, 2002).

2.8.4.2 Chemical degradation of pesticides in soil

Chemical degradation on the other hand includes reactions such as hydrolysis, oxidation-reduction (redox) and ionisation which usually are a pH dependent reaction (Picó & Andreu, 2004). As soil pH increases, microbial activities may decrease but such condition makes chemical degradation more favourable (He *et al.*, 2008).

Chemical degradation usually takes place in the deeper layer of soil where microbial population is limited. Hydrolysis involves the breaking of bonds in a molecule with the presence of water. These reactions will result in the replacement of some functional groups into a hydroxyl groups and it occurs with the presence of hydrogen or hydroxide ion as the catalyst (Frank *et al.*, 2002). Hence, the reaction is strongly dependent to pH.

2.5.4.3 Biodegradation of pesticide in soil

Biodegradation of pesticides in soil has been widely studied (Regitano *et al.*, 2001; He *et al.*, 2008; Malghani *et al.*, 2009; Porto *et al.*, 2011). It involves the breakdown of pesticides by microorganisms such as bacteria and fungi where they use pesticides as an energy source for growth. The breakdown products are usually non-toxic compounds, such as carbon dioxide, water, and minerals. This degradation was usually referred as mineralisation (Regitano *et al.*, 2001; Gosh *et al.*, 2010; Porto *et al.*, 2011).

On the other hand, with the presence of other food sources such as carbon and nitrogen in soil, pesticides may undergo transformation or changes in their molecular structure as a

result of the soil microbial activities. The transformation may result in intermediate products formation which could possibly more toxic and persistent than the parent compound itself (Malghani *et al.,* 2009; Porto *et al.,* 2011).

Microbial degradation rate are influenced by environmental conditions such as temperature, soil pH, moisture, oxygen content, soil organic matter and nutrients as these factors are essential for microorganism's growth (Malghani *et al.,* 2009; Gosh *et al.,* 2010). Alteration of any factors which could affect microorganism activity and population growth in soil may perturb or enhance pesticide degradation rate in soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Reagents

Commercial profenofos, ELAK 45 EC (profenofos 45% w/w), commercial λ-cyhalothrin, ALERT 2.8 EC (λ-cyhalothrin 2.8% w/w), and chlorothalonil, Daconil 2787 (chlorothalonil 50% w/w) were purchased from **the** local market. Profenofos standard (purity 98.2%) was obtained from Sigma Aldrich. λ-cyhalothrin (purity 98.2%) and chlorothalonil (purity 97.5%) standards were obtained from Germany. Acetonitrile, dichloromethane, *n*-hexane, glacial acetic acid, sodium chloride, and anhydrous magnesium sulphate were purchased from J.T. Baker. The silica gel and primary-secondary amine (PSA) were purchased from Merck and Varian, respectively.

3.2 Equipment and Instrumentations

Vegetable sample was chopped and homogenised using a Robot Coupe food chopper. During extraction, sample was shaken using a Vortex-Genie model K-550-GE. Supernatant of extracted sample was obtained by using a Thermo Jouan Model B4i multifunction centrifuge.

An Agilent model 6890 gas chromatography (GC) equipped with a flame photometric detector (FPD) was used for the determination of profenofos. A non-polar fused-silica capillary column, HP5, 1.5 m x 0.53 mm x 1.5 µm obtained from J & W Scientific, USA and was used with nitrogen as carrier gas at a flow of 4.0 mL min^{-1}. A more polar capillary column, DB1701, 15 m x 0.25 mm x 1.0 µm obtained from J&W Scientific, USA was used for the confirmation of pesticides present in vegetable and soil samples. The column

temperature was maintained at 120 °C for 1 min, and then programmed at 30 °C min^{-1} to 150 °C followed by another temperature ramp of 5 °C min^{-1} to 270 °C and held constant at 270 °C for 10 min. The injector and detector temperatures were maintained at 260 °C and 250 °C, respectively. The air and hydrogen gas flows were set at 80 mL min^{-1} and 67 mL min^{-1}, respectively.

An Agilent model gas chromatography GC equipped with electron captured detector (ECD) was used for the analysis of chlorothalonil and λ-cyhalothrin. This instrument was configured with a non-polar fused-silica capillary column, Ultra 1, 25 m x 32 mm and 0.5 µm, obtained from J & W Scientific and employing nitrogen as carrier gas at 1.2 mL min^{-1}. A more polar capillary column, SPB608, 30 m x 0.53 mm x 0.5 µm obtained from J&W Scientific was used for the confirmation of pesticides in vegetable and soil samples. The column temperature was maintained at 120 °C for 0.5 min, then programmed at 10 °C min^{-1} to 180 °C followed by another temperature ramp of 6 °C min^{-1} to 240 °C and subsequently 10 °C min^{-1} to 280 °C and held constant at 280 °C for 12 min. The injector and detector temperatures were maintained at 260 °C and 300 °C. The air and hydrogen gas flow were set at 80 mL min^{-1} and 67 mL min^{-1}, respectively.

3.3 Method development and validation

For recovery studies, homogenized green mustard and soil was fortified with appropriate amount of profenofos, λ-cyhalothrin, and chlorothalonil standard solution to obtain 0.05 mg L^{-1}, 0.1 mg L^{-1} and 0.5 mg L^{-1}, respectively. Each concentration level was prepared in triplicates. Blank samples were also analysed in three replicates as control. After spiking, samples were left in the fume hood (1 h) to allow solvent evaporation and pesticide interaction with the sample.

3.3.1 Extraction of pesticides from green mustard

Green mustard was extracted using an established method (Chai *et al.*, 2012). Homogeneous green mustard (10 g) was weighed in a teflon centrifuge tube. Acetonitrile (20 mL) containing acetic acid (1%) was added to the sample and shaken vigorously by hand (1 min). Sodium chloride (1.5 g) and anhydrous magnesium sulphate (5 g) was added to the sample. The sample was then vortexed (1 min) and centrifuged at 3000 *g* (1 min). The supernatant was transferred into another teflon centrifuge tube and shaken with anhydrous magnesium sulphate (3 g), followed by vortex mixing and centrifuged at 3000 *g* for 1 min.

3.3.2 Extraction of pesticides from soil

Profenofos, λ-cyhalothrin and chlorothalonil residue in soil was extracted using an established method (Chai *et al.*, 2014). Homogeneous soil sample (10 g) was weighed in a teflon centrifuge tube. Acetonitrile (15 mL) containing acetic acid (1 %) was added to the sample and hand shaken vigorously (1 min). Sodium chloride (1.5 g) and anhydrous magnesium sulphate (6 g) was added to the sample. The sample was then shaken with a

vortex (1 min) and centrifuged at 2500 *g* (1 min). The supernatant was transferred into labelled test tubes for clean-up.

3.3.3 Extract clean-up for λ-cyhalothrin and chlorothalonil analysis

Vegetable and soil extract (2 mL) was transferred into a beaker and left to evaporate until dryness. The extract was then eluted with 2 mL of hexane (4 mL): dichloromethane (1 mL), and was leached through deactivated silica gel (0.2 g) packed in a 2 mL glass Pasteur pipette with cotton wool at the bottom. The extract was then eluted again with another 2 mL of hexane (1 mL): dichloromethane (1 mL). Sample extract was left to dry under room temperature, make-up with n-hexane (2 mL) and injected into GC-ECD for quantification.

3.3.4 Extract clean-up for profenofos analysis

For profenofos clean-up, vegetable and soil extract (2 mL) was directly leached through primary secondary amine, PSA (0.2 g) packed in a 2 mL glass Pasteur pipette with cotton wool at the bottom. It was then left to dry under room temperature. Sample was then make-up with acetone (2 mL) and injected to GC-FPD for pesticide quantification.

3.4 Lab incubation study

1.5 mL of 50 mg L^{-1} individual pesticide standard was spiked onto 10 g soil samples prepared in 330 individual vials. Each types of pesticide were prepared in triplicates with 1 sample blank for each batch of samples. Spiked samples were vortexed for 1 min and left in fume hood for 1 h. Samples were covered with aluminium foil, prick on top to allow aeration and incubated under 25 °C in the dark. Water content was monitored weekly and added accordingly to the sample weight lost. Samples were harvested on day 1, 3, 9, 15, 25, 30, 50, 70, 85 and 100 and analysed by using the soil extraction method as described earlier.

3.5 Field experiments (parameter of soil physicochemical properties examined)

This study was conducted at Agriculture Research Centre, Semongok (N01º23'05.9", E110º19'44.7') in October, 2012 for wet season and August, 2013 for dry season. The soil is classified as clayey red-yellow podzolic soil (typic paleudults, very fine, mixed and isohyperthermic) formed from sedimentary rock (Soil Survey Staff, 1999). Top soil physicochemical properties were adapted from Chai *et al.*, 2010 (Table 3.1).

Table 3.1 Physicochemical properties of Semongok top soil at 0-20 cm depth (Chai *et al.*, 2010).

Parameter	Semongok top soil physicochemical properties
pH[a]	4.8
% carbon[b]	2.20
% clay[b]	23.1
% silt[b]	29.6
%fine sand[b]	9.8
% coarse sand[b]	37.6
Moisture content	

[a] pH was determined in 0.01 M $CaCl_2$ in a 1:1 soil:water suspension.

[b] Mass percentage of carbon determined by dry combustion.

[c] Moisture content was determined by oven drying and gravimetric analysis.

The study was conducted in net house (N.H) and open field (O.F). The size of each plot was approximately 15 m x 15 m with sixteen beds measuring about 5.2 m x 1.2 m each (in N.H) and 5.2 m x 1.2 m (in O.F). Prior to planting, the vegetable beds were cleared, tilled, and chicken manure was incorporated into the soil at 0.5 kg m^{-2}. The green mustard was sprayed three times. The first spraying was carried out one week after seeds broadcasting and the second spray was performed 1 week later and the final spray was on the 21st day after seeds were broadcasted. Three pesticides formulation used for this study were profenofos, ELAK 45 EC (profenofos 45% w/w), λ-cyhalothrin, ALERT 2.8 EC (λ-cyhalothrin 2.8% w/w), and chlorothalonil, Daconil 2787 (chlorothalonil 50% w/w). Each pesticide application was carried out in three replicates (three beds) and three remaining beds were kept aside as control. Climatic data was obtained from the local meteorological department throughout the experimental period.

3.6 Sampling of green mustard and soil

For each site, green mustard samples (1 kg) were collected randomly from 12 beds treated in triplicates with profenofos, λ-cyhalothrin and chlorothalonil. 3 untreated beds were also sampled as blank. Sample was collected 2 h after the final pesticide application (21 days after seeds broadcasting) and the sample was indicated as day 0. Sampling was carried out at 9 a.m on the following days (day 1, 3, 5, 7, 10, 14 and 22). Field temperature and humidity recorded during sampling is shown in Appendix XXI. Green mustard sample was taken to the laboratory where the roots were removed. Samples of green mustard treated with specific pesticides were combined, chopped and mixed homogenously.

Similarly, 0.5 kg of the surface soil sample at 2 cm depth was collected between the green mustard plants using a clean spade. The soil sample from the three plots with similar pesticides formulation were combined and mixed homogenously prior to pesticides residue analysis. Debris and stones were also removed from the soil. The plant and soil samples were weighed (10 g each replicate), packed and stored in a freezer at -20 ℃ when they could not be analysed immediately.

CHAPTER 4

RESULTS AND DISCUSSION

Method validation was performed prior to field trials. As the objective of this study was to monitor and verify the compliance with MRLs, the method used in this study was validated to ensure its quality, consistency and sensitivity to reliably determine the residues likely to present in green mustard and soil studied at or around the MRL (EURACHEM, 1998). Method validation was carried out to determine selectivity, linearity, matrix effect, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision of the simplified QuEChERS method for profenofos, λ-cyhalothrin and chlorothalonil determination in green mustard and soil.

4.1 Method validation of pesticides in green mustard

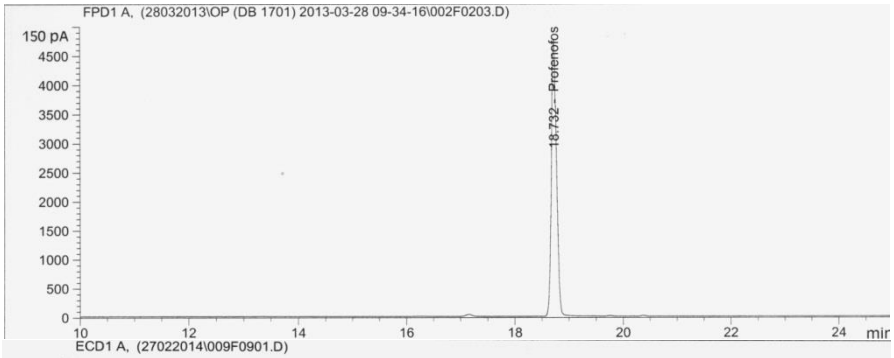
A linear response in the appropriate concentration range is essential to achieve good analytical results of pesticide residue analysis (SANCO, 2013). Linearity was therefore tested for each of the three pesticides. Linearity of the method was evaluated by analysing the standard solutions prepared in different matrices. Analysis was carried out in triplicate within the concentration range of 0.01 to 5 mg kg^{-1}. Good linearity was achieved with a correlation coefficient of 0.997, 0.999 and 0.996 for profenofos, λ-cyhalothrin, and chlorothalonil, respectively.

Individual standard of profenofos, λ-cyhalothrin and chlorothalonil was prepared in green mustard and soil matrix. The individual standards were analysed using GC. The retention time for profenofos, λ-cyhalothrin and chlorothalonil were 18.73, 8.52, and 4.75 min respectively (Figure 4.1). The chromatograms did not show the presence of any matrix

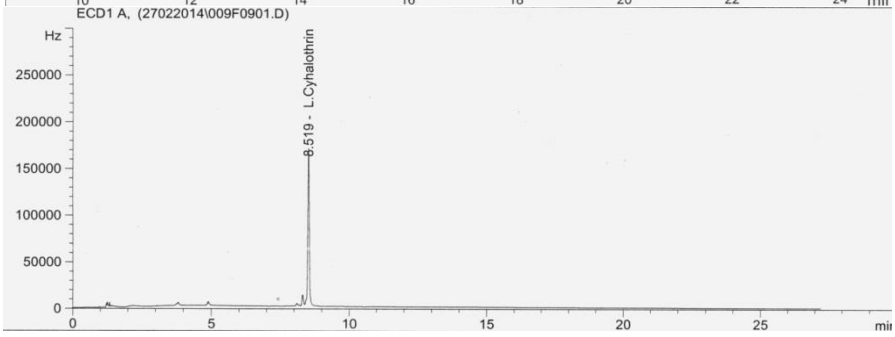
interference from unwanted compounds. Selectivity of the method was assessed by comparing the chromatogram of blank green mustard samples with the corresponding spiked samples. Blank extract of green mustard did not show any interference with the targeted compounds (Figure 4.1).

Limit of detection (LOD) of profenofos, λ-cyhalothrin and chlorothalonil in this study was estimated by six replicate analyses of the lowest-level calibration standard and calculating 3.36 times the standard deviation of the determination results. Limit of detection for profenofos, λ-cyhalothrin and chlorothalonil in green mustard was 0.0061 mg kg^{-1}, 0.0069 mg kg^{-1}, and 0.0077 mg kg^{-1}, respectively. Limit of quantification (LOQ) were defined as the concentration giving a signal to noise ratio of 3 and 10 (EURACHEM, 1998; Kurz *et al.*, 2008). By comparing the responses with the baseline noise in this study, LOQ of the method was 0.001 mg kg^{-1} for the three pesticides.

a)



b)



c)

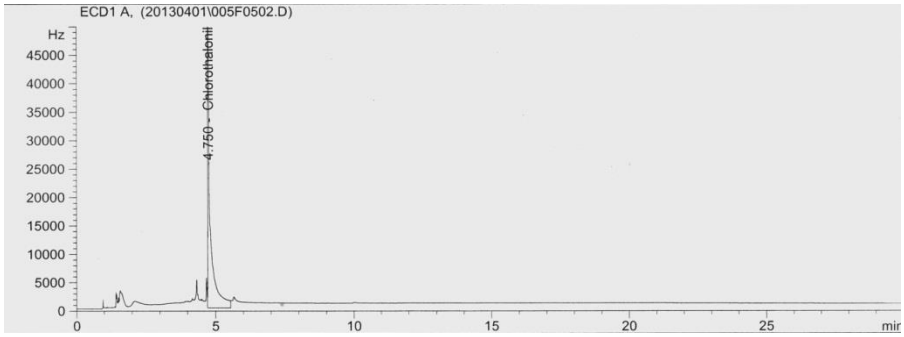


Figure 4.1 Chromatogram of 1 mg L^{-1} standard solution corresponding to profenofos (a), λ-cyhalothrin (b) and chlorothalonil (c) prepared in the matrix extract of green mustard.

Accuracy and precision were estimated by means of recovery experiments (n = 3). The accuracy was expressed by the recovery (%) of spiked samples (Wu *et al.*, 2014; Liu *et al.*, 2012). Method precision was determined by repeatability studies of the method and expressed as relative standard deviation (RSD) values. The acceptable value of RSD for repeatability was ≤ 20% (SANCO, 2013). Blank pesticides free green mustard sample was fortified with a corresponding amount of profenofos, λ-cyhalothrin and chlorothalonil

standard solutions at different expected concentration levels of 0.5, 0.1, and 0.05 mg kg^{-1} and analysed as in the methodology described earlier. Recoveries obtained for profenofos, λ-cyhalothrin and chlorothalonil in green mustard fortified at 0.5, 0.1, and 0.05 mg kg^{-1} are shown in Table 4.1.

Table 4.1 Recovery of profenofos, chlorothalonil and λ-cyhalothrin fortified with 3 different concentration levels of 0.5, 0.1, and 0.05 mg kg^{-1} in green mustard

Pesticide	Spiking level (mg kg^{-1})	Recovery % (RSD, % n=3)
Profenofos	0.5	102.5 (2.4)
	0.1	85.4 (9.3)
	0.05	83.1 (1.7)
λ-cyhalothrin	0.5	97.7 (13.3)
	0.1	93.8 (13.1)
	0.05	81.7 (1.4)
Chlorothalonil	0.5	91.0 (11.3)
	0.1	88.4 (3.2)
	0.05	82.6 (1.5)

The recoveries obtained for profenofos, λ-cyhalothrin and chlorothalonil were in the acceptable range of 70-120% in accordance to analytical method quality control and validation procedures for pesticide residues analysis in food and feed (SANCO, 2013). Lower recoveries were obtained for lower concentration level (0.05 mg L^{-1}) for the three pesticides in green mustard. The mean RSD obtained in this study was < 15%, which is lower than the acceptable value of RSD for repeatability of < 20% (SANCO, 2013).

4.2 Method validation of pesticides in soil

Recoveries of profenofos, λ-cyhalothrin and chlorothalonil in soil fortified at concentration level of 1 and 0.5 mg kg^{-1} are shown in Table 4.2. The recoveries obtained for profenofos were 91.3 and 90.3%, while λ-cyhalothrin was 83.3% and 82.6% for 1 and 0.5 mg kg^{-1}, respectively. Chlorothalonil gave recovery of 82.6% for 1 mg kg^{-1} and 80.6% for 1 and 0.5 mg kg^{-1}. The recoveries of profenofos, λ-cyhalothrin and chlorothalonil were in the acceptable range of 70-120% with RSD < 9% (SANCO, 2013). Chai *et al.,* (2014) reported similar range of recoveries obtained for profenofos and λ-cyhalothrin in mineral soils. Blank soil samples were analysed to assess selectivity and matrix effect. Results obtained showed no interference with the targeted compound.

Table 4.2 Recoveries of profenofos, λ-cyhalothrin and chlorothalonil in Semongok mineral soil fortified with 0.5 and 1.0 mg L^{-1}.

Pesticide	Spiking level (mg L^{-1})	Recovery % (RSD, %, n=3)
Profenofos	0.5	90.3 (3.21)
	1.0	91.3 (2.08)
λ-cyhalothrin	0.5	82.6 (4.51)
	1.0	83.3 (8.18)
Chlorothalonil	0.5	80.6 (2.08)
	1.0	83.0 (2.52)

Injudicious use of pesticides by farmers led to the presence of unwanted residue in food crops produced which may pose risks to human health (Sapbamrer & Hongsibsong, 2014; Juraske *et al.,* 2009; Bhanti & Taneja, 2007). Besides, extensive violation of pesticides usage may also cause deleterious effects to the environment (Bhanti & Taneja, 2007;

Mariän *et al.,* 2003). Pesticides fate and behaviour varies greatly upon the rate and frequency of application, pesticides formulation, crops morphology and weather conditions (Sapbamrer & Hongsibsong, 2014). Therefore, pesticides fate and behaviour need to be evaluated individually on specific crops and under specific environmental conditions through field trials and controlled environment study under laboratory condition.

4.3 The field dissipation of profenofos, λ-cyhalothrin and chlorothalonil on green mustard under humid tropical condition

A field study on the dissipation of three pesticides namely the profenofos, λ-cyhalothrin and chlorothalonil in green mustard and soil was conducted at Agriculture Research Centre, Semongok, Sarawak. As climate conditions such as sunshine, rainfall, surrounding temperature and relative humidity were important in this study; meteorological data was collected throughout the experimental period.

During dry season, the maximum air temperature recorded was 35 °C, while the lowest temperature recorded was 24 °C (Table 4.3). Rainfall was recorded daily at 8 a.m. The average rainfall during this study was 12.03 mm during dry season with daily rainfall frequency of 15 out of 22 days. Relative humidity was ranged from 40-80% under both N.H and O.F, whilst the average daily sunshine measured was 4.5 h.

Table 4.3 Sunshine (h), rainfall (mm) and average temperature (°C) data collected during the experimental period.

Day	Dry Season			Wet season		
	Sunshine (h)	Rainfall (mm)	Mean T (°C)	Sunshine (h)	Rainfall (mm)	Mean T (°C)
0	7.6	0	29.5	4.75	0	28.8
1	1.4	0.9	29.6	1.60	20.9	27.3
2	8.4	45.1	26.0	4.40	0	28.3
3	5.0	0	29.1	3.05	0.8	28.0
4	7.6	0	29.2	4.95	0	28.9
5	2.5	0	28.5	1.25	38.7	28.1
6	9.7	0.3	27.4	1.45	25.3	27.2
7	5.7	3.1	28.5	1.80	2.8	26.2
8	8.0	35.9	28.2	3.40	6.5	28.3
9	7.7	0	28.9	4.05	10.9	28.9
10	6.4	0	28.9	0.40	1	26.4
11	8.9	0	29.4	0.80	18.2	28.1
12	7.6	40.5	29.5	4.05	0	28.7
13	0.0	15	29.7	0.00	11.9	27.5
14	0.0	91.3	23.0	0.25	10.4	27.3
15	0.4	5	24.3	2.45	3	26.8
16	2.5	4.8	26.7	5.95	32.4	28.6
17	0.0	8.3	27.6	5.35	0	28.4
18	3.7	0	26.0	2.00	0	27.4
19	4.3	32.9	27.1	4.85	39.3	28.8
20	3.4	7.4	28.0	3.95	50.5	28.8
21	0.0	13.6	26.3	2.15	3.8	25.2
22	3.0	13.6	27.2	3.95	22.2	28.0

During the wet season, highest air temperature recorded was 34.5 °C, whilst the lowest was 24 °C. Relative humidity ranged from 40-80%. The average rainfall recorded was 11.80 mm with daily frequency of 17 out of 22 days. Frequency of rainfall during the wet season was apparently higher than dry season. However, statistically the amount of rainfall during dry and wet season has no significant difference. Average daily sunshine recorded was 2.9 h, which was lower than that of dry season (4.5 h). In terms of temperature, the dry season has higher average temperature compare to wet season. Statistically, the amount of sunshine and temperature of both seasons were indifferent (Appendix XIII).

The dissipation rate of the three pesticides chosen in this study was expected to vary among the two seasons (dry and wet) as well as under the two cultivation systems used (N.H and O.F). As the three pesticides have different physico-chemical properties, the dissipation rate may vary accordingly. Profenofos has high solubility and high vapour pressure compare to λ-cyhalothrin and chlorothalonil. Therefore, it was postulated to dissipate faster than the other two pesticides.

A study on profenofos dissipation in tropical soil by Ngan and associates (2005) had proved that profenofos has short half-life in soil, therefore profenofos is suspected to dissipate fast in Semongok soil. λ-cyhalothrin on the other hand has lower vapour pressure and water solubility compare to profenofos and chlorothalonil. It has high affinity towards soil and organic matter. Therefore it is envisaged to be more persistent in soil compare to profenofos. In plant, it should dissipate with a slower rate compare to profenofos. Chlorothalonil vapour pressure and solubility in water lies in between profenofos and λ-cyhalothrin. It was therefore suspected to dissipate faster than λ-cyhalothrin but slower than profenofos in both green mustard and soil.

4.3.1 Field dissipation of profenofos insecticide in green mustard

4.3.1.1 During dry season

The dissipation of profenofos in green mustard was analysed by plotting residue concentration against time as shown in Figure 4.2. The initial concentration of profenofos in green mustard cultivated under N.H and O.F were 8.35 and 7.14 mg kg^{-1}, respectively. Within the first 24 h following the final application, 37-44% profenofos had dissipated in green mustard under N.H and O.F, respectively (Appendix I). The dissipation may be due to the volatilisation of profenofos attributed to the high surrounding temperature (29 °C) and longer sunlight radiation (7.6 h). As volatilisation is correlated with the specific vapour pressure of a substance, profenofos was suspected to dissipate *via* this route due to its considerably high vapour pressure (Guth *et al.*, 2004; Fantke & Juraske, 2013).

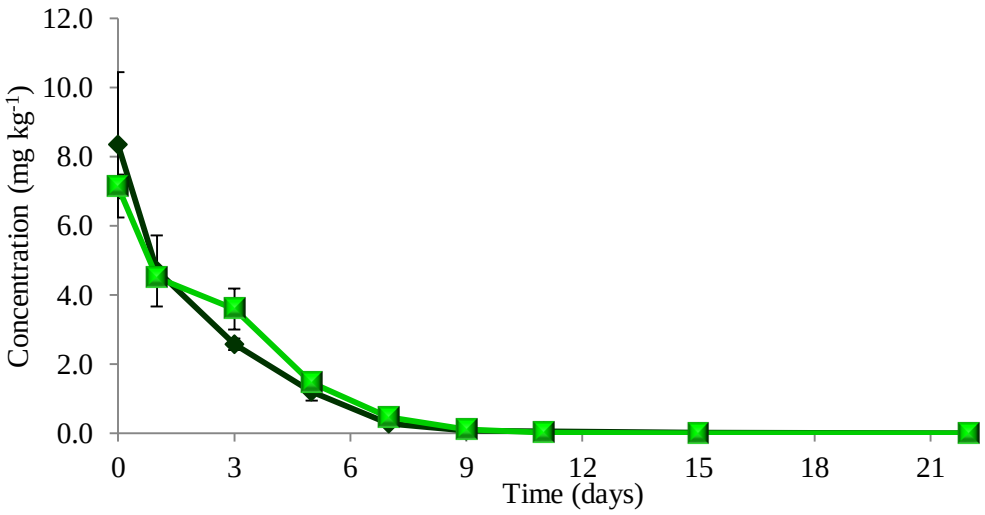


Figure 4.2 The dissipation curves of profenofos residue in green mustard cultivated under N.H (◆) and O.F (◪) cultivation systems during the dry season. Vertical lines represent standard deviation (n=3).

On day 3, 50-69 % of the initial concentration lost under both N.H and O.F. Precipitation (45.1 mm) and 8.4 h of sunlight on day 2 has suspected to contribute to the 45% reduction of profenofos residue under N.H, from 4.70 mg kg^{-1} on day 1 to 2.57 mg kg^{-1} on day 3. Meanwhile in O.F., only 20 % dissipation was observed (from 4.50 mg kg^{-1} in day 1 to 3.59 mg kg^{-1} in day 3). Temperature under N.H may have elevated as a result of heat accumulation due to the hermetic condition of N.H (Talekar *et al.*, 2003). Higher dissipation observed under N.H was therefore suspected to be due to the elevated temperature. Besides, as N.H has higher relative humidity compare to O.F, rapid dissipation of profenofos. These two factors increased profenofos solubility, and thus contribute to its fast disappearance from green mustard in N.H (Katagi, 2004; Fantke & Juraske, 2013).

On day 7, profenofos concentration declined to 0.27 mg kg^{-1} under N.H and 0.46 mg kg^{-1} in O.F. From day 3-7, there was no occurrence of rainfall. Daily temperature ranged from 27.6–29.2 °C with sunshine availability up to 9 h. This has led to 83% residue concentration reduction under N.H and 50% reduction in O.F. Besides, rapid growth of green mustard plant within the first week may have also contributed to the dilution of profenofos residue by plant growth. As the green mustard grows larger, the plant weight will increase, causing decreased in observed profenofos concentration even if there is no degradation occurred (Metwally *et al.*, 1997).

The progression of time after last profenofos application resulted in more dissipation of profenofos residues. The last detectable residue (0.01 mg kg^{-1}) was on day 15, under N.H. However, no residue was detected in green mustard under O.F condition. It showed profenofos dissipated completely in O.F after 15 days. Although the residue was still

detectable in N.H, it complied with the national tolerance level of 0.01 mg kg^{-1} for profenofos in green mustard (Legal Research Board, 2012).

4.3.1.2 During wet season

Similar dissipation trend was also observed for profenofos during wet season, where the residue showed exponential declining trend against time (Figure 4.3). The initial profenofos concentration was 6.60 mg kg^{-1} under N.H and 4.72 mg kg^{-1} in O.F. Compare to dry season, small amount of residue has dissipated within 24 h after application in wet season. Only 26% and 0.4% of initial profenofos residue dissipated under N.H and O.F, respectively (Appendix II). This may be due to reduced sunlight which was only 4.75 h during wet season, thus reducing the effect of photodegradation and volatilisation (Talekar *et al.*, 2003).

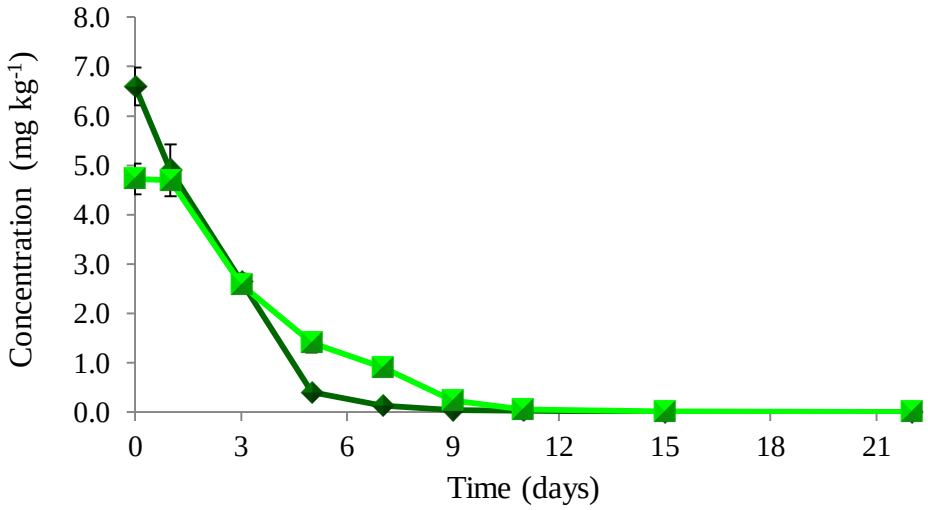


Figure 4.3 The dissipation curves of profenofos residue in green mustard cultivated under N.H (◆) and O.F (■) cultivation systems during the wet season. Vertical lines represent standard deviation (n=3).

Rapid dissipation of profenofos was observed from day 1-5, following 20.9 mm rainfall on day 1 and 38.7 mm on day 5 (Table 4.3). During this period, about 94% of the initial concentration of profenofos dissipated under N.H, whilst only 70% of the initial concentration of profenofos dissipated in O.F. Profenofos has high water solubility and high vapour pressure (Ngan *et al.*, 2005). Because of its high solubility in water, profenofos was envisaged to dissipate more rapidly under O.F condition with intense rain compare to N.H. However rapid dissipation also occurred under N.H where rainfall intensity was reduced. This could be due to the higher temperature under N.H during rainfall. N.H temperature was found to be 5-10% higher than open field in the afternoon. The hermetic condition and limited air flow leading to temperature accumulation in N.H during rainfall (Talekar, 2003). Higher surrounding temperature may accelerate pesticides dissipation (Fantke & Juraske, 2013). Besides, growth dilution could be another factor which contributed to rapid profenofos dissipation in green mustard from day 1-5.

On day 7, total dissipation under N.H was 98% of the initial concentration (from 6.60 mg kg^{-1} to 0.14 mg kg^{-1}). However in O.F, total dissipation was only 81% (from 4.72 mg kg^{-1} to 0.91 mg kg^{-1}). Faster dissipation was observed under N.H compared to O.F. Frequent rainfall during wet season contributed to higher humidity (70% r.h) under N.H condition. High humidity and higher temperature under N.H during wet season may have contributed to rapid dissipation of profenofos in green mustard. The dissipation may occur *via* volatilisation and dilution (Fang *et al.*, 2006). Apart from that, the dissipation of profenofos under both systems may partially due to growth dilution as rapid growth of green mustard was observed within the 7 days.

Slower dissipation in the later stage (day 9-22) could be due to the tenancy of the profenofos residues which remained at trace level (< 0.1 mg kg^{-1}) in green mustard, as lower concentration of pesticides is hard to be removed by means of physical dissipation (wash off and volatilisation). On day 15, profenofos residue was still detectable under N.H (0.01 mg kg^{-1}) and O.F (0.02 mg kg^{-1}). The residue however dissipated completely on day 22, where none was detected under both N.H and O.F (Appendix II).

In summary, profenofos showed a biphasic dissipation pattern in green mustard during both dry and wet season under N.H and O.F. Rapid dissipation took place from day 1-7 followed by a slower dissipation from day 9-22. Rapid dissipation was due to several factors such as photodegradation, volatilisation, wash-off by rain, growth dilution factor and plant metabolism (Fantke & Juraske, 2013). Slower rate of dissipation was suspected to be due to plant metabolism (Zablotowicz *et al.*, 2005). Similar observation of biphasic dissipation was also reported by Nath and associates (2005) on profenofos dissipation in okra where faster dissipation took place in phase I (day 0-1) followed by a slower dissipation in phase II (day 1-7).

4.3.1.3 The dissipation half-life of profenofos in green mustard

The dissipation of profenofos in green mustard under N.H and O.F cultivation system was fitted into the first-order kinetic equation, $C_t = C_{t0}\, e^{-kt}$, employing a non-liner least-squares regression analysis of residue concentration against time, where C_t is the concentration at time t, C_{t0} is the initial concentration and k is the degradation rate (Sun *et al.*, 2012). The degradation rate k and the correlation coefficient (R^2) were obtained from regression analysis of the concentration plotted against time (day) after application for each curve (Chaves *et al.*, 2007). The slope parameter was used as the least squares estimate of k. The

half-life ($t_{1/2}$, day) was estimated from equation, $t_{1/2} = \ln 0.5\ k^{-1}$ (Table 4.4). For dry season, calculated half-life of profenofos in green mustard was 0.71 and 0.66 day for N.H and O.F cultivation system, respectively. For wet season, the half-life was slightly longer i.e. 1.28 days for N.H and 1.76 days for O.F.

Table 4.4 Half-life ($t_{1/2}$) of profenofos in green mustard under N.H and O.F during the dry and wet season.

Season/ Cultivation system	**Regression equation**	**R^2**	**Half-life ($t_{1/2}$) (day)**
Dry season			
N.H	$C= 8.35\ e^{-0.9667t}$	0.9736	0.72
O.F	$C= 7.14\ e^{-1.0493t}$	0.9046	0.66
Wet season			
N.H	$C= 6.60\ e^{-0.543t}$	0.9750	1.28
O.F	$C= 4.72\ e^{-0.393t}$	0.9280	1.76

Dissipation of profenofos was observed to be more rapid during dry season, resulting in shorter half-life compare to wet season. This phenomenon was probably due to rapid volatilisation and photodegradation of profenofos on plant surfaces (Fantke & Juraske, 2013). Dry season has longer sunlight and higher surrounding temperature. Furthermore, rainfall during dry seasons may have caused faster dissipation of profenofos from green mustard. As the half-life of profenofos during dry and wet season has a very small difference, therefore they are statistically indifferent (Appendix XIX).

Profenofos also showed slightly longer half-life in N.H (0.72 day) in dry season compare to O.F (0.66 day). Longer sun irradiation and higher surrounding temperature has led to faster dissipation of profenofos in green mustard under O.F conditions. This implies that

volatilisation and photodegradation are a dominant process in profenofos residue degradation in green mustard (Fantke & Juraske, 2013). Shading under N.H was suspected to impede profenofos dissipation and led to its longer half-life compare to O.F. Similarly to seasonal factors, cultivating system used gave no significant effects onto profenofos residue dissipation. As the amount of residue was in trace level, the differences were statistically insignificant (Appendix XIV).

During wet season profenofos half-life was observed to be longer in O.F compared to N.H. The result proved profenofos dissipation was faster under higher surrounding temperature compare to direct rainfall effect. The effect of rainfall was more pronounced in the earlier stage after pesticides application. In the later stage, profenofos dissipation occurred mainly *via* other patyhways such as photodegradation, volatilisation and growth dilution (Metwally *et al.,* 1997). Mean temperature during wet season was lower than dry season. However in N.H cultivation system trapped warm air led to heat accumulation especially during rainfall. While in O.F, heat was dispersed by the movement of air and thus leading to lower surrounding temperature (Talekar *et al.,* 2003). As surrounding temperature increased, profenofos volatility and solubility may also increase and thus increasing wash-off efficiency in N.H.

Half-life of profenofos in this study (0.72-1.76 day, Table 4.4) was comparable to those reported for non-leafy vegetables such as okra, which is 1.35 days, 2.7 days for bitter gourd (Nath *et al.*, 2005; Gupta *et al.*, 2012) 1.84 days for hot pepper, 1.74 days for sweet pepper, and 1.96 days for eggplant (Radwan *et al.,* 2005). Half-life of profenofos in/on plant or vegetation may vary not only according to the climatic conditions and types of

formulation used or dosage applied, but it also depends on the growth rate, plant matrices, sizes and surface areas (Metwally *et al.,* 1997; Juraske *et al.,* 2009; Gupta *et al.*, 2012).

Initial deposit of profenofos in bitter gourd planted under subtropical conditions was 1.29 mg kg^{-1} which was lower than the initial deposit detected in this study (4.72-8.35 mg kg^{-1}). It is because bitter-gourd has smaller surface area and waxy peel, while green mustard has larger surface area. Therefore, green mustard can trap more pesticide compared to bitter gourd, leading to higher initial concentration detected. However, the residues persisted for up to 15 days in both green mustard and bitter gourd. Therefore it could be concluded that profenofos dissipation rate in vegetable under humid tropical conditions was faster than the subtropical condition. Due to high solar intensity and high rainfall frequency in a tropical climate, half-life of a pesticide is shorter compare to temperate region (Chai *et al.,* 2008).

Apart from profenofos, other insecticide (λ-cyhalothrin) which belong to the Py group is predicted to show similar pattern of dissipation on green mustard during the two seasons. However, the dissipation rate may be different compare to profenofos with respect to its physico-chemical properties. It has lower water solubility and lower vapour pressure compare to profenofos, therefore, λ-cyhalothrin is suspected to dissipate mainly *via* photo degradation as the Pys are relatively photosensitive compare to OP and other group of pesticides (Shukla & Omkar, 1998).

4.3.2 Field dissipation of λ-cyhalothrin insecticide in green mustard

4.3.2.1 During dry season

The dissipation curves of λ-cyhalothrin in green mustard under N.H and O.F cultivation system during the dry season is shown in Figure 4.4. Initial deposit of λ-cyhalothrin was 0.41 and 0.38 mg kg^{-1} under N.H and O.F, respectively. Lower initial concentration of λ-cyhalothrin in green mustard compare to profenofos was due to the lower active ingredient (a.i) percentage in the commercial formulation of λ-cyhalothrin used. λ-cyhalothrin exhibited similar dissipation trend with profenofos. It was observed to dissipate exponentially against time with rapid dissipation took place during the first 7 days after application followed by slower dissipation from day 9-22. Within the first 24 h following the final application, 61% of the initial concentration under N.H and 47% under O.F have dissipated (Appendix III).

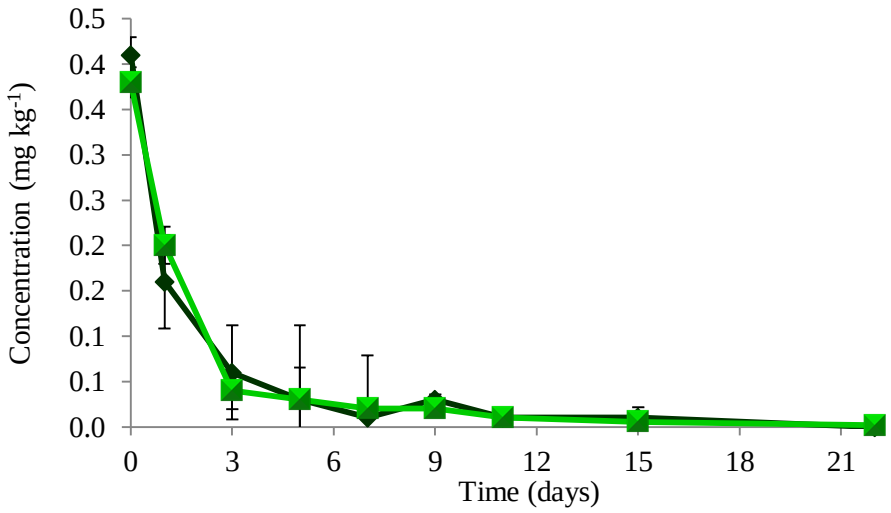


Figure 4.4 The dissipation curves of λ-cyhalothrin residue in green mustard cultivated under N.H (◆) and O.F (◪) cultivation systems during the dry season. Vertical lines represent standard deviation (n=3).

In day 0, high (29.5 °C) surrounding temperature and long sunshine (7 h) was suspected to cause rapid dissipation of λ-cyhalothrin within the first 24 h. λ-cyhalothrin has low stability under sunlight compared to profenofos. A number of literatures have mentioned its photosensitivity and its high possibility to degrade *via* photodegradation (Hill & Inaba, 1991; Fernandez-Alvarez *et al.*, 2007; He *et al.,* 2008). However, λ-cyhalothrin has a very low vapour pressure compare to profenofos (He *et al.*, 2008). Dissipation *via* volatilisation or evaporation may have also took place but could be in a very low rate compare to volatilisation of profenofos.

The dissipation of λ-cyhalothrin is suspected to be mainly due to breakdown *via* photodegradation. Fernandez-Alvarez and associates (2007) proposed several possible mechanisms occur during the photodegradation of λ-cyhalothrin. These mechanisms include decarboxylation, reductive dehalogenation and ester or other bond cleavage initiated mainly by the presence of reactive species generated from UV radiation of sunlight (Katagi, 2004; Fernandez-Alvarez *et al.,* 2007). The metabolites formed may undergo further degradation into non-toxic compounds such as CO_2 and water (He *et al.,* 2008).

The dissipation of λ-cyhalothrin took place on day 1-3 under N.H conditions resulted to 63% residue loss (Appendix III). While in O.F, λ-cyhalothrin residue concentration declined from 0.2 to 0.04 mg kg^{-1}, resulting to 80% loss. Rainfall in day 2 (45.1 mm) seemed to dislodged quite a big amount of λ-cyhalothrin residue from the green mustard. The washed-off effect was more apparent in O.F with higher percentage of concentration loss observed. Greater λ-cyhalothrin removal in O.F might be due to direct rainfall wash-off, whilst the netting reduced rainfall intensity under N.H leading to less wash-off effect.

Observation made from day 3-7 under N.H showed that λ-cyhalothrin dissipated from 0.06 to 0.01 mg kg^{-1}, which accounted to 83% residue loss. While in O.F, the residue declined from 0.04 to 0.02 mg kg^{-1}, accounting for only 50% reduction in concentration. Within the period of day 3-7, there was no rainfall, high temperature (about 27 to 29 °C) and long sunshine (5 to 9.7 h) was observed. Therefore λ-cyhalothrin dissipation within this period could be attributed to photodegradation and growth dilution, as rapid growth of green mustard was observed especially under N.H.

From day 7-11, λ-cyhalothrin residue remained unchanged under N.H (0.01 mg kg^{-1}), while in O.F, the concentration decrease slightly from 0.02-0.01 mg kg^{-1}. Slower rate of dissipation observed within this period was also observed for profenofos. Although there was a rainfall on day 8 (35.9 mm), λ-cyhalothrin residue concentration on green mustard was intact. A possible explanation for this could be the retention of a trace amount of λ-cyhalothrin residue within the plant cuticular wax (Fife & Nokes, 2002) in green mustard under both N.H and O.F.

During dry season, λ-cyhalothrin was observed to persist up to 22 days in green mustard in spite of continuous rainfall within day 12-21. The possibility of trace amount of λ-cyhalothrin to be held within the plant matrices may have reduced the rainfall wash-off effect (Xu *et al.*, 2002). This can be prove further by Monadjemi *et al.*, (2011) where lipophilic pesticides has high tendency to diffuse into cuticular waxes or inner part of plant which prevent its removal *via* direct rainfall wash-off.

4.3.2.2 During wet season

During wet season, average surrounding temperature and sunshine availability was relatively lower compared to dry season. However, rainfall frequency was much higher which may induce more pesticides wash-off. The dissipation curve was obtained by plotting λ-cyhalothrin residue concentration against time (Figure 4.5). From the dissipation curve, λ-cyhalothrin was observed to dissipate exponentially against time demonstrating similar trend as observed for profenofos in wet season.

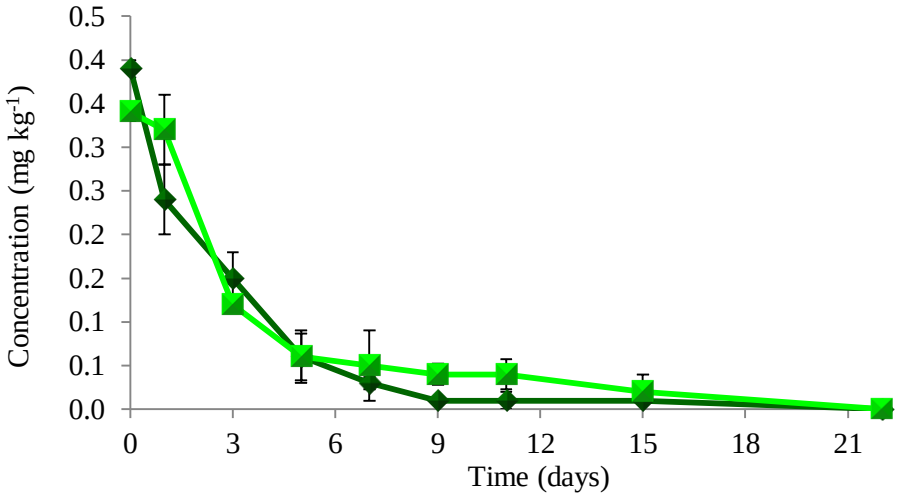


Figure 4.5 The dissipation curves of λ-cyhalothrin residue in green mustard cultivated under N.H (◆) and O.F (◪) cultivation systems during the wet season. Vertical lines represent standard deviation (n=3).

The initial concentration of λ-cyhalothrin in green mustard was 0.32 and 0.39 mg kg^{-1}, lower than profenofos due to its lower active ingredients (a.i) percentage (2.8% w/w) compare to profenofos (45% w/w). On day 1, λ-cyhalothrin residue concentration decreased up to 39% in wet season (Appendix IV). There was no rainfall within the first 24 h. Sunshine (4 to 7 h) and high temperature (29.3 °C) was assumed to induce photodegradation of λ-cyhalothrin in green mustard (He *et al.*, 2008). This is because λ-

cyhlothrin is a photo labile pesticide and their main degradation pathway was ascribed to photodegradation (He *et al.,* 2008).

From day 1-3, λ-cyhalothrin concentration declined from 0.24 to 0.15 mg kg^{-1} accounted to 38% dissipation under N.H. whilst in O.F, 63% of the residue detected in day 1 has dissipated (Appendix IV). Rainfall on day 1 has resulted to more λ-cyhalothrin dissipation in O.F compared to N.H. The netting shaded and reduced rainfall intensity under N.H and thus not much dissipation was observed in green mustard planted under N.H (Metwally e*t al*., 1997). As λ-cyhalothrin is a hydrophobic pesticide, intense rainfall would be needed to effectively dislodge it from the leaf surface (He *et al.,* 2008). This explained the difference of λ-cyhalothrin dissipation under N.H and O.F during the period of day 1-3.

λ-cyhalothrin continued to dissipate rapidly from day 3-7, where 80% residue decline from 0.15 to 0.03 mg kg^{-1} under N.H. However, only 58% diminution observed in O.F (from 0.12 to 0.05 mg kg^{-1}). During the study, continuous rainfall occurred on day 3-7. However, N.H showed more rapid dissipation compared to O.F, which was in contradiction with observation made in day 1-3 earlier. The most likely reasons for these could be due to a number of processes which has led to λ-cyhalothrin dissipation, such as rain wash-off (which appeared to be less effective later after the application), growth dilution (as rapid growth of green mustard plant was observed within this period) and hydrolysation which is more favourable under highly humid condition with higher temperature such as in N.H (Metwally *et al.,* 1997; He *et al.,* 2008). Heat accumulation under N.H might accelerate several processes involved in λ-cyhalothrin dissipation (Fantke & Juraske, 2013). Therefore, more rapid dissipation had occurred in N.H compare to O.F on day 3-7.

On day 7-11, rainfall (21.2 mm) however, showed no apparent effect towards λ-cyhalothrin dissipation in green mustard. It was due to the fact that most λ-cyhalothrin deposits on green mustard following application can be washed off easily shortly after application, compare to the remaining residue which is more persistent (Xu *et al.*, 2008). Besides, the possibility of λ-cyhalothrin residue being held by plant cuticular matrix suggests it may not easily wash-off by rain (Fife & Nokes, 2002; Xu *et al.*, 2008).

On day 12-22, the dissipation of λ-cyhalothrin occurs very slowly until no residue detected on day 22. Terminal residue was detected on day 15, 0.01 mg kg^{-1} under N.H and 0.02 mg kg^{-1} in O.F. Intense and continuous rainfall was not effectively remove remaining residue in green mustard. As the remaining residue was suspected to be held within the plant matrices, diminution of λ-cyhalothrin might have occurred *via* plant metabolism which involved cellular enzymatic reaction (Juraske *et al.*, 2008).

4.3.2.3 The dissipation half-life of λ-cyhalothrin in green mustard

The dissipation of λ-cyhalothrin in green mustard fitted the first-order kinetics. He *et al.*, (2008) has pointed out that the photodegradation of λ-cyhalothrin followed first-order kinetic behaviour. Half-life (day) of λ-cyhalothrin estimated from equation, $t_{1/2} = \ln 2/k$ in green mustard for dry season was 1.18 days and 1.25 days for N.H and O.F cultivation systems. While for wet season, calculated half-life of λ-cyhalothrin in N.H and O.F cultivation system was 2.51 and 3.69 days.

Table 4.5 Half-life ($t_{1/2}$) of λ-cyhalothrin on green mustard under N.H and O.F conditions during dry and wet season.

Season/ Cultivation system	Regression equation	R^2	Half-life ($t_{1/2}$) (day)
Dry season			
N.H	$C= 0.41\ e^{-0.5872t}$	0.9450	1.18
O.F	$C= 0.38\ e^{-0.5532t}$	0.8762	1.25
Wet season			
N.H	$C= 0.39\ e^{-0.2763t}$	0.8908	2.51
O.F	$C= 0.32\ e^{-0.1879t}$	0.8921	3.69

Half-lives of λ-cyhalothrin on green mustard during wet season were observed to be longer than those observed during dry season. This showed that higher air temperature, solar radiation and hence volatilisation and photodegradation appeared to increase pesticides dissipation in vegetable (Chai *et al.*, 2008; Katagi, 2004; Fantke & Juraske, 2013). λ-cyhalothrin has shorter half-lives (1.18 and 2.51 days) under N.H compare to O.F (1.25 and 3.69 days) in both wet and dry seasons. This suggested that the dissipation rate of λ-cyhalothrin was faster in N.H compare to O.F. However the half-life differs in a very small degree. Therefore, the dissipation rate of λ-cyhalothrin under N.H and O.F has no significant difference (Appendix XV)

Faster rate of dissipation for λ-cyhalothrin under N.H appeared to be related to the lipophilicity property of λ-cyhalothrin which was explained by its high octanol-water coefficient (log K_{ow}) value. Lipophilic pesticides tend to be retained by the fatty plant surface tissue which favours the partitioning of the active ingredients into inner plant cells where enzymatic transformation took place (Juraske *et al.*, 2008). Higher relative humidity

under the N.H allowed this partitioning to occur as higher air humidity improves the adsorption affinity of λ-cyhalothrin to plant surfaces (Fantke & Juraske, 2013). Therefore, the dissipation of λ-cyhalothrin could also be due to plant metabolism and growth dilution in addition to rainfall and sunlight radiation (Metwally *et al.,* 1997).

Results obtained from this study were similar to those reported by Fan *et al.*, (2013) for λ-cyhalothrin applied on *pak choi*, spinach and lettuce during summer in Beijing were in the range of 0.6-1.3 days. Other similar studies also reported on the degradation of λ-cyhalothrin in fresh green and black tea leaves planted in Tamil Nadu, India during dry season. The study reported half-life of λ-cyhalothrin varied from 2.8 to 3.5 days under dry condition which is longer than half-life obtained for λ-cyhalothrin in present study (Seenivasan & Muraleedharan, 2009).

Dry season in subtropical condition has lower humidity, surrounding temperature and sunlight intensity, compare to dry season under tropical conditions. Therefore, half-life of λ-cyhalothrin reported by Seenivasan and Muraleedharan (2.8-3.5 days) was longer than those reported in this study for dry season, which was 1.16 days. Albadri *et al.,* (2012) have also reported longer half-life of λ-cyhalothrin in tomato (5.7 days) compared to our findings. It can be further evidence that for a tropical climate with high solar intensity and high rainfall frequency, the half-lives of λ-cyhalothrin reported in this study were shorter (Chai *et al.,* 2008).

4.3.3 Field dissipation of chlorothalonil fungicide in green mustard

4.3.3.1 During dry season

Apart from profenofos and λ-cyhalothrin, chlorothalonil dissipation behaviour in green mustard was also studied. Chlorothalonil is a widely used fungicide in vegetables cultivation. During dry season, chlorothalonil showed similar exponential dissipation pattern with profenofos and λ-cyhalothrin (Figure 4.6). The initial concentration of chlorothalonil in green mustard cultivated in N.H and O.F was 10.03 and 13.22 mg kg^{-1} respectively, higher than profenofos and λ-cyhalothrin. This is due to its higher a.i percentage (50% w/w) in the commercial formulation used. Within the first 24 h, only 6.1% of the initial deposit under N.H has dissipated, while in O.F 47% of the initial residue has dissipated (Appendix V).

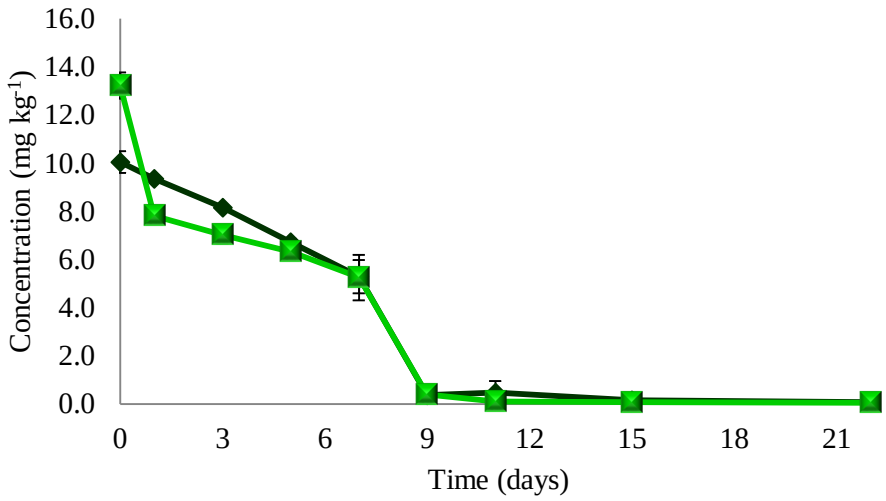


Figure 4.6 The dissipation curves of chlorothalonil residue in green mustard cultivated under N.H (◆) and O.F (■) cultivation systems during dry season. Vertical lines represent standard deviation (n=3).

In the first 24 h the surrounding temperature was 29.5 °C with sunshine available for 7.6 h. higher dissipation rate was observed for O.F compare to N.H within the first 24 h. Similar trend was also observed for profenofos, where O.F showed greater dissipation percentage.

This showed that pesticides with high vapour pressure such as profenofos and chlorothalonil can easily volatilise under O.F condition with higher temperature and sunlight intensity. The shading under N.H reduced sunlight intensity, thus minimised photodegradation.

On day 1-3, N.H cultivation system showed the reduction in concentration from 9.36-8.17 mg kg^{-1} (accounted to 13% loss). While in O.F, chlorothalonil residue loss was slightly lower with only 10% has dissipated (7.08-7.03 mg kg^{-1}). An occurrence of rainfall at day 2 (45.1 mm) and low surrounding temperature impeded chlorothalonil dissipation from green mustard. However, N.H cultivation system showed greater dissipation of chlorothalonil compared to O.F. Similarly to profenofos during dry season, heat accumulation under N.H and hence temperature rise caused an increase in volatilisation rate of chlorothalonil with respect to its vapour pressure (Fantke & Juraske, 2013).

On day 3-7, chlorothalonil concentration declined to 5.29 mg kg^{-1} under N.H and 5.24 mg kg^{-1} in O.F, which accounted to 35 and 25% reduction of concentration (Appendix V). No occurrence of rainfall resulted in high surrounding temperature (27.6-29.2 °C) with daily sunshine up to 9 h. Therefore chlorothalonil dissipation was ascribed to physical losses *via* volatilisation. Although for some pesticides volatilisation may only effective within the first few days after application, chlorothalonil volatilisation may have been occurred in a low rate and prolonged up to several days (Leistra & Van Den Berg, 2007). Besides, rapid growth of green mustard within the first week could also contribute to reduction in chlorothalonil residue concentration *via* growth dilution (Valverde-Garcia *et al.*, 1993).

On day 7-11, chlorothalonil demonstrated rapid dissipation. Chlorothalonil dissipated from 5.29 to 0.47 mg kg^{-1} under N.H condition on day 11 which accounted to 91% loss. In O.F, the concentration declined from 5.24 to 0.1 mg kg^{-1}, which accounted to 98% loss. Rapid dissipation of chlorothalonil within this period was likely due to high rainfall at day 8 (35.9 mm). Since large amount of chlorothalonil were still remaining on green mustard, rainfall wash-off effect was more apparent. Fife and Nokes (2002) have contended that greater amount of deposits were more readily dislodgeable by rain compare to smaller residue as smaller residue tend to be held within the cuticular matrixes of plant.

On day 11-22, chlorothalonil dissipation continued with a considerable rapid rate. There was a frequent rainfall event from day 12-19, resulted to 197.8 mm precipitation. However the chlorothalonil residue persisted until day 22, where 0.08 mg kg^{-1} and 0.04 mg kg^{-1} residues were still detected in the green mustard sample under N.H and O.F respectively. Fife and Nokes (2002) have contended that after readily removable chlorothalonil was removed from the foliage by rain, the additional rainfall event did not remove the remaining chlorothalonil residue. This would explain the remaining residue of chlorothalonil in present study despite of continuous rainfall within the period. The residue of chlorothalonil exceeded national tolerance level of 0.01 mg kg^{-1} for green mustard (Legal Research Board, 2012).

In summary the dissipation trend of chlorothalonil in green mustard during dry season showed different trend with profenofos and λ-cyhalothrin under similar climatic factor, where slower initial dissipation was observed followed by rapid dissipation towards the end of experiment. Chlorothalonil, however, was found to be more persistent than profenofos and λ-cyhalothrin, where the terminal residue detected at day 22 was

considerably high. It could be due to the high percentage of chlorothalonil active ingredients (a.i) in the formulation used in this study (50% a.i) compared to profenofos (45% a.i) and λ-cyhalothrin (2.8% a.i). In addition, the dissipation rate of the 3 pesticides studied showed a statistically significant difference which was presumed to be due to the variation of a.i in the formulation used (Appendix XIII).

4.3.3.2 During the wet season

Chlorothalonil dissipation was also investigated during the wet season, where average temperature and sunshine availability observed was lower compared to dry season. Since rainfall has been reported to show greater removal effect for fungicides deposits on plant compared to the other climatic factors such as sunlight and temperature, chlorothalonil should dissipate more rapidly during wet season (Bruhn & Fry, 1982; Fife & Nokes, 2002). Chlorothalonil was observed to dissipate exponentially against time during wet season with rounder exponential curve compare to dry season.

Similar dissipation trend was also observed in profenofos and λ-cyhalothrin, where rapid dissipation was observed in the first 7 days after the last application, followed by slower dissipation observed on day 9-22 (Figure 4.7). Initial concentration of chlorothalonil in green mustard cultivated under the N.H and O.F were 11.72 and 11.33 mg kg^{-1} respectively. Within the first 24 h, there was not much dissipation observed under both N.H and O.F. Only 6.2% of chlorothalonil residue has dissipated under N.H and 5.7% has dissipated in O.F (Appendix VI). The surrounding temperature was lower (27 °C) compare to dry season with very short sunshine hour (4.75 h) leading to only small amount of residue loss.

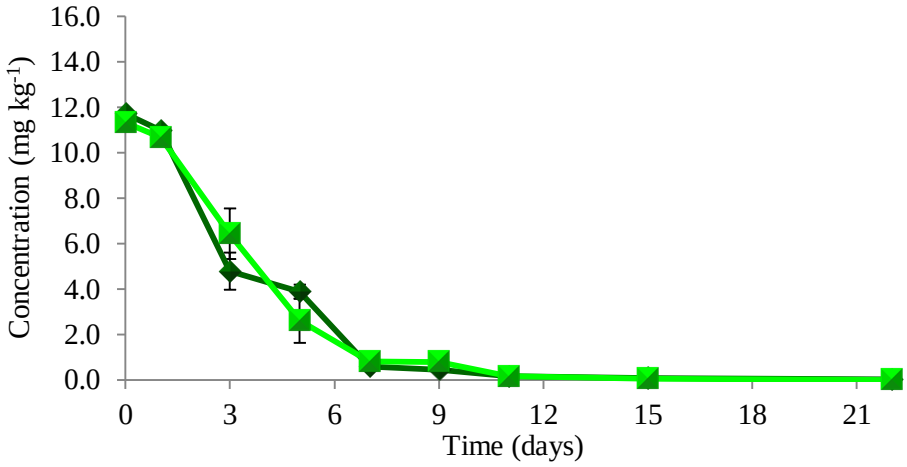


Figure 4.7 The dissipation curves of chlorothalonil residue in green mustard cultivated under N.H (◆) and O.F (◪) cultivation system during wet season. Vertical lines represent standard deviation (n=3).

In day 1-3, 60% of the initial residue dissipated under N.H and 43% in O.F. There was an occurrence of rainfall (20.9 mm) a day after last spray which has led to rapid loss of chlorothalonil. Similar trend was also observed in profenofos dissipation during wet season. Therefore, rapid dissipation of profenofos and chlorothalonil under N.H compare to O.F demonstrated the role of high surrounding temperature in accelerating the dissipation of pesticides with high vapour pressure. The effect of rainfall on chlorothalonil removal however was found to give greatest impact if rainfall occurs on the day of application or soon after, and the effect of rain declines with times (Bruhn & Fry, 1982). This phenomenon explained why the dissipation of chlorothalonil from green mustard in the first 3 days during wet season showed higher decline in residue compared to dry season.

On day 3-7, chlorothalonil residue declined from 4.79 to 0.59 mg kg^{-1} in N.H which accounted to 88% residue loss. While in O.F, the residue declined from 6.44 to 0.82 mg kg^{-1} resulting to 87% loss of residue. It was observed that the dissipation rate under N.H and O.F was almost similar, and there was no significant difference of dissipation rate under N.H and O.F (Appendix XVI). The dissipation of chlorothalonil within this period could be due to a number of processes such as volatilisation, rain wash-off, chemical degradation and growth dilution as plant growth observed within this period was rapid (Mariän *et al.*, 2003). Continuous rainfall with a total of 64 mm precipitation could have washed some of the remaining residues on green mustard which has led to higher rate of dissipation.

Chlorothalonil dissipation on day 7-22 occurred with a slower rate in spite of frequent rainfall within the period. This observation is another example of the persistency of trace level compound in plant as a result of diffusion into the cuticular waxes (Juraske *et al.*, 2009). This observation was also in agreement with those observed by Fife and Nokes, (2002) where the remaining deposit of chlorothalonil on tomatoes was difficult to remove with more rain because it was held within the cuticular waxes. Another similar pattern has been reported, for chlorothalonil loss from potatoes (Bruhn & Fry, 1982) and captan loss from apple and apple leaves (Xu *et al.*, 2008).

The effect of rainfall on chlorothalonil dissipation from green mustard may vary accordingly depending on rainfall intensity, duration and frequency. Fife and Nokes, (2002) observed mean chlorothalonil residues loss were higher for plants exposed to a rainfall intensity of >25 mm h^{-1}compared to plants exposed to intensity level of 13 mm h^{-1}. However, for longer rainfall duration periods, the influence on foliar chlorothalonil lost

because the length of rainfall period became more prominent, and the effect of rainfall intensity was no longer significant (Fife & Nokes, 2002).

4.3.3.3 The dissipation half-life of chlorothalonil in green mustard

The dissipation of chlorothalonil in green mustard under N.H and O.F cultivation systems were fitted into the first-order kinetic equation. Calculated half-life ($t_{1/2}$, day) of chlorothalonil in green mustard for dry season were 1.07 and 0.93 day under N.H and O.F cultivation systems, respectively. During wet season, the half-life under N.H and O.F cultivation systems were 2.21 and 2.01 days (Table 4.6).

Table 4.6 Half-life ($t_{1/2}$) of chlorothalonil in green mustard under N.H and O.F conditions during dry and wet season.

Season/ Cultivation system	**Regression equation**	**R^2**	**Half-life ($t_{1/2}$) (day)**
Dry season			
Net house	$C= 10.03\ e^{-0.6760t}$	0.8787	1.03
Open field	$C= 13.22\ e^{-0.8038t}$	0.8887	0.86
Wet season			
Net house	$C= 11.72\ e^{-0.3133t}$	0.9525	2.21
Open field	$C= 11.33\ e^{-0.3448t}$	0.9782	2.02

Half-lives of chlorothalonil in green mustard during wet season were observed to be longer than in dry season. This suggested that the dissipation rate of chlorothalonil was faster during dry season. Hypothesis testing on the effect of season onto the dissipation of chlorothalonil however showed that growing seasons have no significant effect onto chlorothalonil residue dissipation from green mustard (Appendix XVI). This could be due

to a very small difference in terms of residue concentration and half-life of chlorothalonil between both seasons. In terms of cultivating system used, both seasons show shorter half-life (0.86 and 2.02 days) under O.F condition compare to N.H (1.03 and 2.21 days) in spite of some observations made earlier indicating more rapid dissipation under the N.H. During dry season, the mean air temperature and sunlight radiation was high especially in O.F, as in N.H, sunlight radiation was observed to be reduced (20%) due to shading. Statistically, there was no significant effect of cultivating systems used onto chlorothalonil half-life on green mustard (Appendix XIV).

Chlorothalonil dissipation half-lives have been reported in other crops such as cucumber, tomatoes, spring cabbage, peppers and banana leaves (Valverde-Garcia *et al.,* 1993; Zhang *et al.,* 2007; Kurz *et al.,* 2008; Chaves *et al.,* 2008). Kurz *et al.,* (2008) has reported chlorothalonil half-life of 8.8 days for tomato and 1.6 days for cucumbers. Zhang *et al*., (2007) on the other hand has reported that the half-life of chlorothalonil in spring cabbage was 1.8 days. In banana leaves, chlorothalonil half-life was 3.9 days (Chaves *et al.*, 2008). While in another finding, cucumbers, peppers and cherry tomatoes grown in a commercial green-house showed varied half-life of 5.3, 7.3 and 11.5 days, respectively (Valverde-Garcia *et al.,* 1993).

The variation in chlorothalonil dissipation half-lives from the previous study can be attributed to different species cultivated, dosage applied and experimental site climatic conditions (Kurz *et al.,* 2008). As expected, faster dissipation showed by shorter half-lives of chlorothalonil was observed under the humid tropical condition compare to sub-tropical and temperate condition with lower temperature, lower sunlight radiation and lower rainfall frequency (Chai *et al.,* 2008).

4.3.5 Half-life variation of profenofos, λ-cyhalothrin and chlorothalonil in green mustard

Field dissipation of profenofos, λ-cyhalothrin, and chlorothalonil in green mustard varied depending on the physico-chemical properties of pesticides which thereafter manipulated the fate and behaviour of these compounds under different climatic conditions (Juraske *et al.*, 2013). Highly soluble pesticides for instance would dissipate faster with the presence of rainfall. Apart from that, other competing dissipation processes such as plant penetration, growth dilution and chemical degradation might have also come into play (Leistra & Van Den Berg, 2007).

All three pesticides showed longer half-life under N.H for both dry and wet season (Table 4.7) due to shading (20% reduction in sunlight intensity) under N.H. Photodegradation and volatilisation might be in a lower rate compared to under O.F. Some portions of the residue may be drifted by wind during spraying which has led to the lower initial deposit concentration under O.F. Besides, higher sunlight intensity and wind velocity under O.F might have led to higher lost attributed to photodegradation and volatilisation, thus leading to lower initial pesticides deposit recovered from green mustard compare to N.H.

Table 4.7 Initial deposit and half-life variation of profenofos, λ-cyhalothrin and chlorothalonil in green mustard cultivated under N.H and O.F condition in dry and wet season.

Pesticide	Initial deposit (mg kg^{-1})				Half-life, $t_{1/2}$ (day)			
	Dry season		Wet season		Dry season		Wet season	
	N.H	O.F	N.H	O.F	N.H	O.F	N.H	O.F
Profenofos	8.35	7.14	6.60	4.72	0.72	0.66	1.28	1.76
λ-cyhalothrin	0.41	0.38	0.39	0.34	1.18	1.25	2.51	3.69
Chlorothalonil	10.03	13.22	11.72	11.33	1.03	0.86	2.21	2.02

Profenofos has higher water solubility and vapour pressure compare to λ-cyhalothrin and chlorothalonil (Table 4.8). Therefore profenofos was predicted to show highest rate of dissipation compare to λ-cyhalothrin and chlorothalonil. Results obtained from present study confirmed our prediction where profenofos showed shorter half-life compare to λ-cyhalothrin and chlorothalonil in green mustard during both dry and wet seasons. Other OP insecticides were also reported to have shorter half-life in crops compare to Py insecticides. Acephate and chlorpyrifos (both are OP) have shorter half-life than cypermethrin (Py) in green mustard (Chai *et al.,* 2008).

In terms of seasonal variation, wet season as expected showed longer half-life than dry season. This result showed faster rate of dissipation in crops was due to high surrounding temperature and sunlight intensity which facilitate photodegradation and volatilisation (Chai *et al.,* 2008; Juraske *et al.,* 2013). Between two cultivation systems used, half-lives of the three pesticides however showed some discrepancy and these was suspected to be related to individual pesticides' properties and predominant aspect of their field dissipation. Hypothesis testing however showed that there was no significant difference on

the half-life of the 3 pesticides in green mustard under N.H and O.F (Appendix XVII & XVIII).

Water solubility of profenofos, chlorothalonil and λ-cyhalothrin are 28 mg L^{-1}, 0.81 mg^{-1}, and 0.004 mg L^{-1}, respectively (Table 4.8). The half-lives of pesticides were in the sequence of profenofos < chlorothalonil < λ-cyhalothrin which confirmed the significant effect of rainfall washed-off onto the dissipation rate of these three pesticides. On the other hand, vapour pressure of profenofos, chlorothalonil, and λ-cyhalothrin are in the sequence of 1.24×10^{-1} mPa at 25 °C > 7.62×10^{-5} mPa at 25 °C > 2.00×10^{-7} mPa at 20 °C. The reverse sequence of pesticides average half-life was λ-cyhalothrin (1.25-3.69 days) > chlorothalonil (0.86-2.21 days) > profenofos (0.66-1.76 days).

Table 4.8 Solubility, vapour pressure and octanol-water coefficient of profenofos, λ-cyhalothrin and chlorothalonil.

Pesticide	**Solubility (mg L^{-1})**	**Vapour pressure (mPa)**	**Octanol-water coefficient (log K_{ow})**
Profenofos	28 mg L^{-1} (at 25 °C)	1.24×10^{-1} mPa at 25°C	4.44
λ-cyhalothrin	0.004 mg L^{-1} (at 20 °C)	2.00×10^{-7} mPa at 20°C	7.00
Chlorothalonil	0.81 mg L^{-1} (at 25 °C)	7.62×10^{-5} mPa at 25 °C	2.88

Maximum residue limits (MRLs) of profenofos, λ-cyhalothrin and chlorothalonil in green mustard are 0.01 mg kg^{-1}, 0.20 mg kg^{-1} and 0.01 mg kg^{-1} (Codex Alimentarius FAO/WHO, 2000). Based on the MRLs given, pre-harvest interval (PHI) required for the safe consumption of profenofos, λ-cyhalothrin and chlorothalonil treated green mustard was 15, 3 and 22 days respectively (Table 4.8).

Green mustard has short crop-cycle of 21 days. Repeated application of profenofos and chlorothalonil therefore is not advisable since the tolerable residue concentration allowed on green mustard was 0.01 mg kg^{-1} for both profenofos and chlorothalonil (Legal Research Board, 2012). Besides, the residues persist until up to 15 days for profenofos (0.01-0.02 mg kg^{-1}) and up to 22 days for chlorothalonil (0.01-0.08 mg kg^{-1}). Therefore, harvest at the optimum pre-harvest intervals obtained from this study is not favourable as the plant have been overly matured at the time of harvest.

4.4.1 Profenofos dissipation behaviour in field topsoil

4.4.1.1 During dry season

Apart from evaluating the dissipation of profenofos, λ-cyhalothrin and chlorothalonil in green mustard, the pesticides residue dissipation behaviour in topsoil was also studied. Soil cultivated with green mustard was sampled after the green mustard sample was taken. Based on the chemical properties of each pesticides used in this study, profenofos was envisaged to dissipate faster than λ-cyhalothrin and chlorothalonil in soil. It has high water solubility which may make it prone to leaching and it has high vapour pressure which will enhance its dissipation by volatilisation (Ngan *et al.*, 2005).

Profenofos dissipation in cropped topsoil during dry season is shown in Figure 4.8. Initial concentration of profenofos deposit detected in topsoil was 0.02 mg kg^{-1} in N.H and 0.08 mg kg^{-1} in O.F. On day 1, profenofos residue in topsoil was 35% and 77% higher under both N.H and O.F respectively (Appendix VII). Although there was only small amount of rainfall (0.9 mm) occurred within the 24 h period, the increase of profenofos might probably be due to residue washed-off from the leaf surface following the stint rainfall. Ciglash and co-workers have pointed out the ability of dewfall to dislodged pesticide

deposits from leaf surface onto the ground (Ciglash *et al.*, 2006). Therefore, small amount of rainfall occurred in this study was suspected to be sufficient to dislodge profenofos residue from green mustard onto the topsoil.

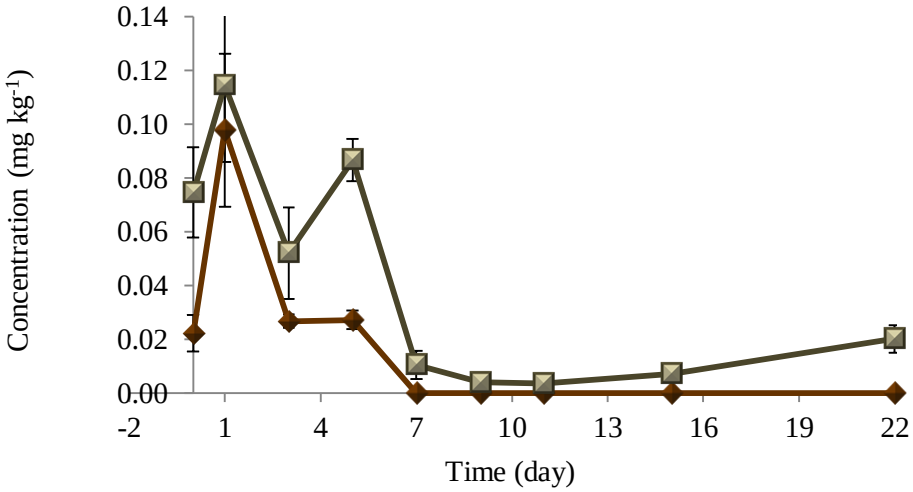


Figure 4.8 The dissipation curves of profenofos in cropped topsoil under N.H (◆) and O.F (▩) cultivation systems during dry season. Vertical lines represent standard deviation (n=3).

Heavy rain on day 2 (45.1 mm) had caused steep drop in the amount of profenofos detected in Semongok topsoil in day 3, where up to 75 % have dissipated under N.H and 54 % in O.F (Appendix VII). As profenofos has high water solubility, it may leach easily into deeper region of soil below the sampling point (Rice *et al.*, 2002; Ngan *et al.*, 2005). This demonstrated that in addition to degradation, leaching is an important route of dissipation for polar pesticides (Chai *et al.*, 2010). Volatilisation of profenofos from soil was not likely to occur in spite of high temperature observed in day 3 (29 °C). Laabs *et al.*, (2002) pointed out that more polar pesticide would not be affected by their high vapour pressure. It is because leaching of pesticides into deeper soil due to rainfall events limit

losses of pesticides *via* volatilisation (Laabs *et al.*, 2002). Therefore, profenofos dissipation in day 3 was suspected to be attributed to rainfall.

Since there was no rainfall occurred in day 3 and day 4, profenofos residue was suspected to dissipate by means of photodegradation or volatilisation in day 5. However, higher concentration was detected in day 5 compared to day 3 (Appendix VII). Increasing residue concentration detected in day 5 could possibly be due to the wick effect, where leached pesticides migrated back up to the soil surface layer as a result of surface evaporation after rainfall (Rice *et al.*, 2002; Taylor, 2005). This effect was more apparent under O.F condition where sunlight intensity was higher leading to 40% increase of the residue detected (Appendix VII). Profenofos however, did not persist longer in Semongok topsoil where in day 7, no residue detected in N.H, while residue concentration in O.F was negligible (0.007-0.001 mg kg^{-1})

4.4.1.2 During wet season

In wet season, higher rainfall frequency was envisaged to enhance profenofos dissipation from the topsoil. Lower surrounding temperature might reduce volatilisation of profenofos from soil and thus impeding its dissipation from soil. In wet season, profenofos however showed similar dissipation pattern as in dry season (Figure 4.9). In dry season, rapid dissipation was due to volatilisation. Rapid dissipation in wet season was suspected to be due to its high water solubility and hence dissipation *via* wash-off by rain. Initial concentration of profenofos deposit detected in topsoil was 0.03 mg kg^{-1} under N.H and 0.08 mg kg^{-1} in O.F. On day 1, an increase in profenofos concentration was observed under N.H condition. However in O.F, profenofos residue concentration decreased (Appendix VIII).

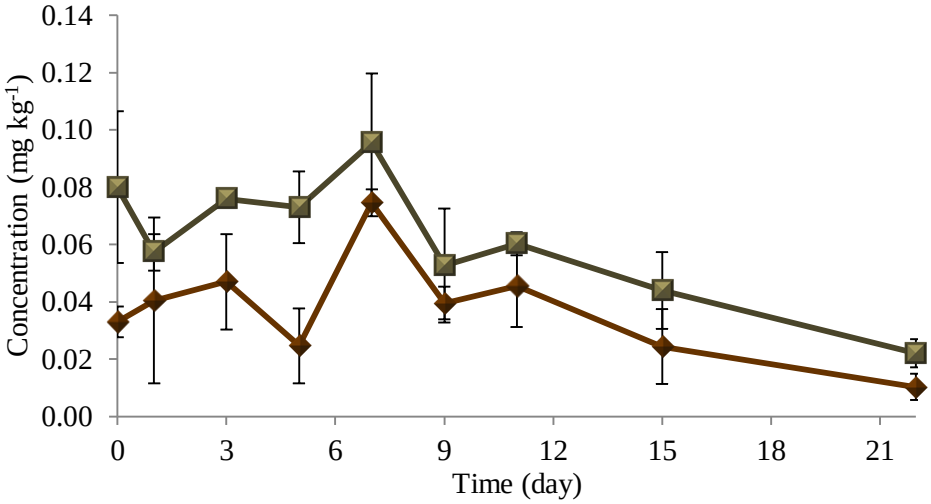


Figure 4.9 The dissipation curves of profenofos in cropped topsoil under N.H (◆) and O.F (■) cultivation systems during wet season. Vertical lines represent standard deviation (n=3).

Profenofos residue in the top soil continued to escalate up to 13% (from 0.04-0.047 mg kg^{-1}) under N.H and 25% in O.F (from 0.05-0.08 mg kg^{-1}) as it is raining on day 1 (20.9 mm). An increase in profenofos concentration in topsoil was likely due to deposit washed-off from green mustard and thus the residues accumulate on topsoil. Simultaneously, apparent concentration losses were also observed in the green mustard on day 1-3 which further affirmed of this observation (Ciglash *et al.,* 2006).

On day 5, profenofos residue in topsoil decreased up to 48% under N.H and 4% in O.F. Small dissipation observed here could partially due to microbial degradation, photodegradation and volatilisation. Under O.F condition, sunlight intensity is higher compare to N.H where net and crops canopy shading reduced the intensity (Hill & Inaba, 1991; Metwally *et al.,* 1997). Shading of the net and crop canopy preserved critical soil moisture under N.H which then enhances soil microbial activity leading to higher dissipation compare to O.F (Hill & Inaba, 1991).

Rainfall on day 5 (38.7 mm) and day 6 (25.3) have led to rapid profenofos dissipation from green mustard. In spite of the downpour event, profenofos residue detected in the top soil in day 7 had increased extensively compared to day 5. Under N.H condition, accumulation of profenofos residue in topsoil was comparable with total residue dissipated from green mustard (Appendix VII & VIII). Similar trend was also observed for top soil in O.F. Profenofos residue was observed to increase from 0.07-0.10 mg kg^{-1} giving up to 24% increase which coincided with 36% dissipation of profenofos from green mustard. This phenomenon was due to residue washed-off from the leaves onto the soil (Ciglash *et al.*, 2006).

On day 9-22, profenofos residue started to decline with time. Profenofos was reported to have short half-lives of 8 days in soil (Ngan *et al.*, 2005; Wan Abdullah *et al.*, 1999). Due to non-correlated field data, profenofos half-life was not calculated in this study. Profenofos however persisted up to 22 days in top soil during wet season where the residues were still detectable at 0.01-0.002 mg kg^{-1} under both cultivation systems. It was suggested that profenofos dissipated faster during dry season with higher mean temperature compare to wet season. Under tropical condition, rapid dissipation is not necessarily caused by enhanced degradation of pesticides but can also be due to higher volatilisation of the substance from soil (Racke *et al.*, 1997). As profenofos has high vapour pressure, remarkably faster dissipation in dry season was suspected.

Study of profenofos dissipation fate and behaviour in tropical soil is scanty. In this study, profenofos was observed to dissipate quickly in soil especially during dry season where surrounding temperature is high. Similar study on the short half-life of profenofos in soil

was also reported (Wan Abdullah, 1999; Ngan *et al.,* 2005) where the dissipation rate was correlated with surrounding temperature and leachability of the compound in soil.

4.4.2 λ-cyhalothrin dissipation behaviour in cropped soil

4.4.2.1 During dry season

Apart from profenofos, λ-cyhalothrin dissipation behaviour in cropped topsoil had also been investigated. λ-cyhalothrin is a hydrophobic insecticide with lower vapour pressure compared to profenofos. Hence, it was envisaged to exhibit slower dissipation in cropped topsoil compared to profenofos. λ-cyhalothrin was reported to degrade in soil *via* photodegradation and microbial degradation (Hill & Inaba, 1991; Laabs *et al.,* 2000; He *et al.,* 2008).

λ-cyhalothrin residue in Semongok cropped topsoil was observed to dissipate very slowly in dry season (Figure 4.10). Initial concentration of λ-cyhalothrin recovered from sampled topsoil was 0.03 mg kg^{-1} under N.H and 0.02 mg kg^{-1} in O.F. Lower initial concentration of λ-cyhalothrin compared to profenofos was due to the lower a.i percentage of the commercial formulation used. Initial dissipation of pesticides under field condition is usually due to initial surface losses *via* evaporation, photolysis and volatilisation (Hill & Inaba, 1991). Surface evaporation may lead to either pesticides loss from soil or accumulation of pesticides on soil surface (Rice *et al.,* 2002). Surface evaporation may have occurred within 24 h after pesticide application where λ-cyhalothrin concentration under N.H decreased while in O.F the concentration increased. This is because higher soil temperature in O.F was suspected to lead to higher evaporation rate.

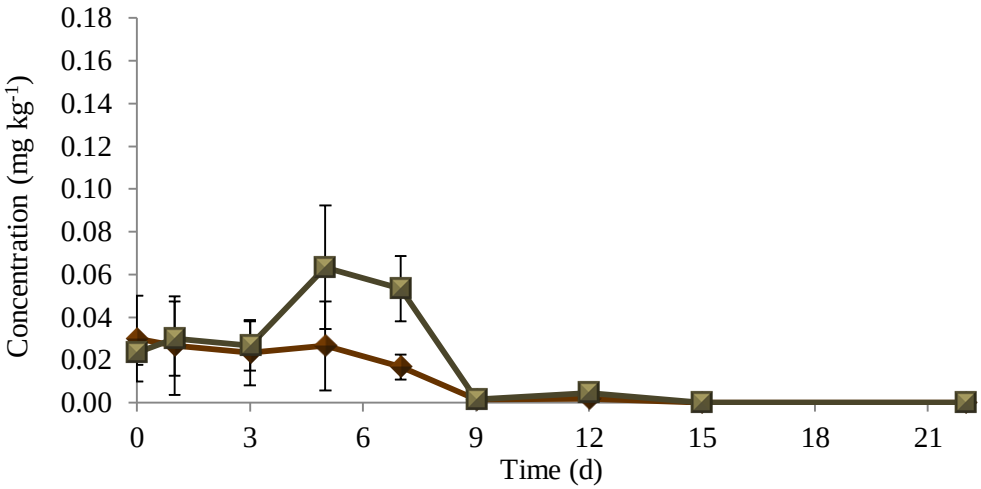


Figure 4.10 The dissipation curves of λ-cyhalothrin in cropped topsoil under N.H (◆) and O.F (■) cultivation systems during dry season. Vertical lines represent standard deviation (n=3).

λ-cyhalothrin residue continued to decline until day 3. As profenofos dissipation within this period was correlated to its high water solubility, insoluble λ-cyhalothrin dissipation was likely due to preferential flow and particle-facilitated transport of its bound residue into deeper layer of soil (Laabs *et al.,* 2002). Surface degradation *via* photodegradation may have also played a role in the dissipation of λ-cyhalothrin residue in O.F (He *et al.,* 2008). Lower sunlight intensity under N.H due to net and crops canopy shading may have lowered the possibility of photodegradation to occur under N.H. Therefore, lower losses were observed under N.H compared to O.F within the first 24 h period.

λ-cyhalothrin residue concentration increased abruptly on day 5 especially in O.F. Increasing λ-cyhalothrin residue in day 5 could be correlated to the 'wick effect' following rainfall in day 2. Wick effect is described as the upward movement of leached residue due to the surface layer as a result of surface evaporation after rainfall (Rice *et al.,* 2002). Besides, residue wash-off from the leaf surface may have also contributed to accumulation

of λ-cyhalothrin in topsoil on day 5. Similar pattern was also observed for profenofos and chlorothalonil during dry season, which was also suspected to be due to surface evaporation after rainfall.

On day 7-22, λ-cyhalothrin concentration in Semongok topsoil started to decline slowly. Slower dissipation of pesticides 7 days after application was frequently referred to as microbial degradation (Hill & Inaba, 1991). Microbial degradation may have taken place in soil over the experimental period but due to high relevance of physical processes such as photodegradation, adsorption and leaching, the effect of microbial degradation reported in the literature was completely masked and could not be quantified in the earlier stage of dissipation (Ciglash *et al.*, 2006).

4.4.2.2 During wet season

Wet season has lower surrounding temperature compared to dry season. Therefore the dissipation rate of λ-cyhalothrin was suspected to be lower than dry season. Under N.H, 0.14 mg kg^{-1} λ-cyhalothrin residue was recovered in the sampled cropped top soil and 0.08 mg kg^{-1} was detected in O.F. λ-cyhalothrin showed exponential dissipation pattern (Figure 4.11). 24 h following final application, λ-cyhalothrin residue decreased to 0.08 mg kg^{-1} under N.H which accounted to 42% loss. While in O.F, λ-cyhalothrin concentration decreased to 0.06 mg kg^{-1}, which was 25% of the initial concentration detected on day 0.

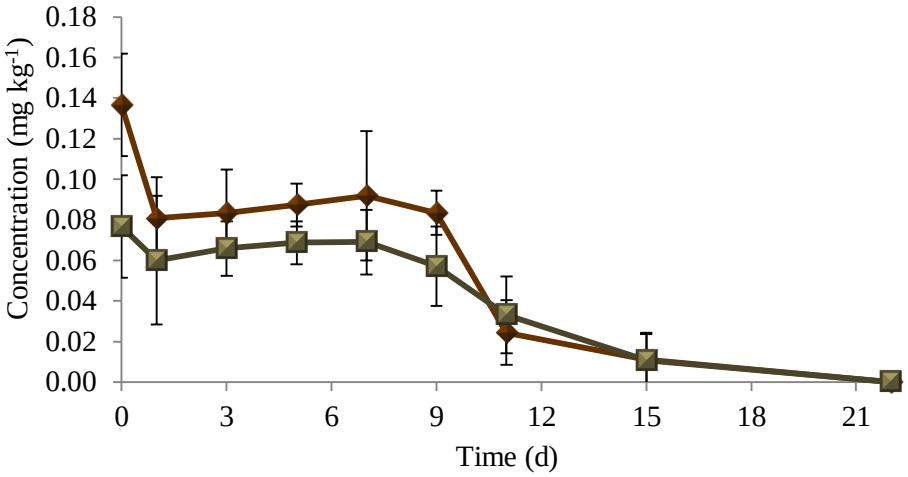


Figure 4.11 The dissipation curves of λ-cyhalothrin in cropped topsoil under N.H (◆) and O.F (■) cultivation system during wet season. Vertical lines represent standard deviation (n=3).

More dissipation under N.H compared to O.F in the first 24 h was probably due to higher soil microbial activity under N.H. Higher soil activity was associated with optimum soil moisture and temperature (Hill & Inaba, 1991). Under N.H, soil was more shaded and thus it took longer time to dry after rain compared to O.F. Therefore wetter soil under N.H has enhanced microbial activity and increased degradation of λ-cyhalothrin. Evaporation might have occurred, however lower surrounding temperature may have caused evaporation process to be limited compare to in dry season.

λ-cyhalothrin concentration detected on day 3 was higher than day 1. λ-cyhalothrin concentration increased continuously until day 7. Profenofos has also reached the highest peak in day 7 during wet season. This was could be due to the 'wick effect' following rainfall on day 5-6 (Rice *et al.*, 2002). Besides, an additional concentration of λ-cyhalothrin could be washed-off from the green mustard leading to accumulation on

topsoil. High percentage of λ-cyhalothrin residue dissipation from green mustard on day 7 confirmed the possible washed-off effect of the rainfall.

After reaching the highest peak on day 7, λ-cyhalothrin concentration in Semongok topsoil declined steadily and completely dissipated on day 22. It was reported that λ-cyhalothrin was susceptible towards photodegradation, which was likely to occur during the first 3 days, followed by chemical and microbial degradation, which were likely to occur at the later stage (Laabs *et al.,* 2000; He *et al.,* 2008). As it has high affinity towards soil, therefore it was suspected to bind with soil particles which may inhibit its degradation (He *et al.,* 2008). Apart from that, no sunlight has led to slower dissipation of λ-cyhalothrin as photodegradation was inhibited.

4.4.3 Chlorothalonil dissipation behaviour in soil

4.4.3.1 During dry season

Rainfall and cultivation systems used were likely to affect profenofos and λ-cyhalothrin dissipation from topsoil. As profenofos and λ-cyhalothrin showed similar dissipation trend in Semongok cropped topsoil regardless of their different physicochemical properties, chlorothalonil was envisaged to dissipate in a similar way. Chlorothalonil degradation in soil has been widely studied because of the interest in its metabolites formation in soil which was reported to be more stable and toxic than the parent compound itself (Regitano *et al.,* 2001; Putnam *et al.,* 2003; Chaves *et al.,* 2007; Chaves *et al.,* 2008). An example of chlorothalonil metabolites is 4-hydroxychlorothalonil. It was reported to be 30 times more toxic in acute toxicity and more mobile in soil and water than the parent compound itself (Carlos-Rojas *et al.,* 2003).

The depicted chlorothalonil dissipation trend in Semongok cropped topsoil is depicted in Figure 4.12. Chlorothalonil showed non-linear dissipation pattern which could be due to uneven amount of deposit distribution on soil due to green mustard canopy enclosure. Hill and Inaba (1991) highlighted the difficulty to accurately and evenly apply pesticides through a growing crop onto the soil. Variation might have aroused from uneven deposits in different sampling spots on the soil.

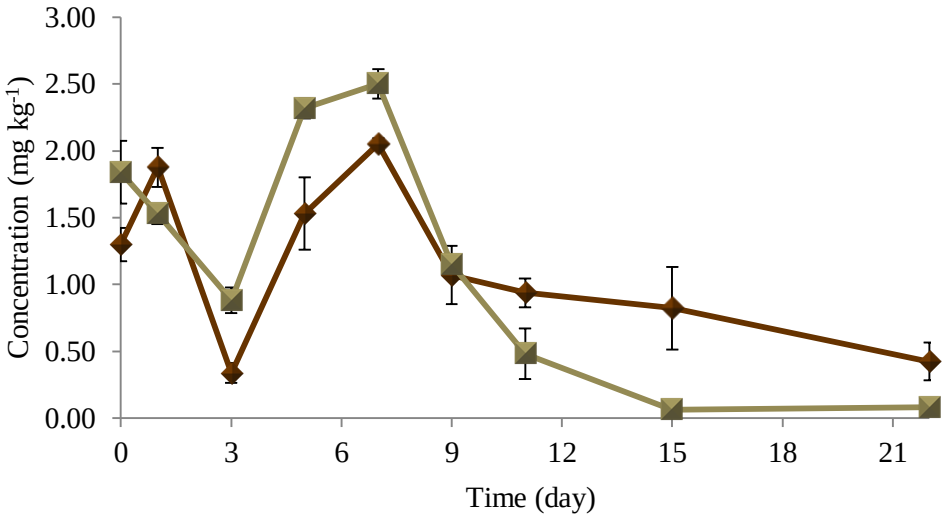


Figure 4.12 The dissipation curves chlorothalonil in cropped topsoil under N.H (◆) and O.F (■) cultivation system during dry season. Vertical lines represent standard deviation (n=3).

Initial concentration of chlorothalonil detected in topsoil was 1.30 mg kg^{-1} in N.H and 1.84 mg kg^{-1} in O.F, respectively. Under N.H, chlorothalonil concentration increase after 24 h leading to higher concentration detected on day 1 compared to day 0. While O.F showed decreasing concentration after 24 h. This trend was in opposite with λ-cyhalothrin where after 24 h, concentration under N.H decreased while in O.F the concentration increased (Figure 4.11). Humid condition under N.H was believed to enhance chlorothalonil degradation. The most possible reason for this phenomenon could be deposited wash-off

from green mustard which led to chlorothalonil accumulation in top soil. Besides, the hermetic condition of N.H limited the chlorothalonil volatilisation (Metwally *et al.*, 1997).

Chlorothalonil concentration showed a steep drop from day 1-3. It was believed as a result of rainfall in day 2. Rainfall was reported to cause steep drop of pesticide residue concentration in topsoil, especially polar pesticides. Rainfall had drawn the pesticides downward into deeper soil layer leading to dissipation from the topsoil layer (Ciglash *et al.*, 2006). As rainfall continued, the concentration continued to decline. Similar to profenofos during wet season, volatilisation of chlorothalonil from soil during this period was not likely to occur as it has leached into deeper layer of soil (Laabs *et al.*, 2002).

On day 5, chlorothalonil residue concentration was increased under both N.H and O.F. Chlorothalonil residue appeared to be drawn upward toward the soil surface which led to residue accumulation in topsoil. Chlorothalonil residue continued to increase on day 7 where 2.05 mg kg^{-1} detected under N.H and 2.50 mg kg^{-1} in O.F. This phenomenon was referred as the 'wick effect' (Rice *et al.*, 2002). In addition to the aforementioned 'wick effect', increasing concentration of chlorothalonil in top soil on day 5-7 may also attributed to chlorothalonil washed-off from the green mustard by a stint rainfall event on day 6. As dewfall was reported to be sufficient enough to dislodge an amount of pesticides from leaf surface, therefore it is possible for the small amount of rain to wash-off chlorothalonil from green mustard residue accumulation on topsoil (Ciglash *et al.*, 2006).

From day 9-22, chlorothalonil declined continuously in topsoil. Heavy downpour on day 8 (35.9 mm) was believed to leach down chlorothalonil into deeper layer of soil and reduced its concentration in topsoil. Apart from leaching, preferential flow may have also caused

rapid dissipation of chlorothalonil from topsoil as chlorothalonil tend to form bound residues in soil (Regitano *et al.,* 2001). Chai *et al.,* (2009) pointed out that top soils with high moisture contents may induced macropore flow which facilitate pesticide migration in solution or as sorbed to particles.

Slow and continuous dissipation of chlorothalonil on day 9-22 could be partially attributed to biodegradation. This is because biodegradation is always correlated to the slower dissipation process of pesticides in soil (Laabs *et al.,* 2002). Soil moisture, soil organic matter content, and soil temperature are the key parameter for the activity of pesticides degrading microbes in soil (Racke *et al.,* 1997; Laabs *et al.,* 2002). Therefore faster dissipation was observed in O.F compared to N.H (Figure 4.12). Linear correlation between soil temperature and mineralisation rate of pesticides in soil reaffirmed the crucial role of temperature in pesticides biodegradation (Kookana *et al.,* 2010).

4.4.3.2 During wet season

During wet season, initial chlorothalonil residue detected in topsoil was much lower compared to dry season. Under N.H, chlorothalonil residue was 0.39 mg kg^{-1} while in O.F chlorothalonil residue was 0.62 mg kg^{-1} (Figure 4.13) Chlorothalonil residue showed steep drop on day 1, which accounted to 61% of the initial concentration loss under N.H from 0.3933 mg kg^{-1} to 0.1500 mg kg^{-1} and 55% loss in O.F, from 0.6200 mg kg^{-1} to 0.2850 mg kg^{-1}. High surrounding temperature (28.8 °C) may have caused rapid chlorothalonil losses *via* volatilisation (Katayama *et al.,* 1994). Besides, formation of bound residue may have started within the 24 h after last application as higher temperature accelerates pesticides binding to soil (Potter *et al.,* 2001).

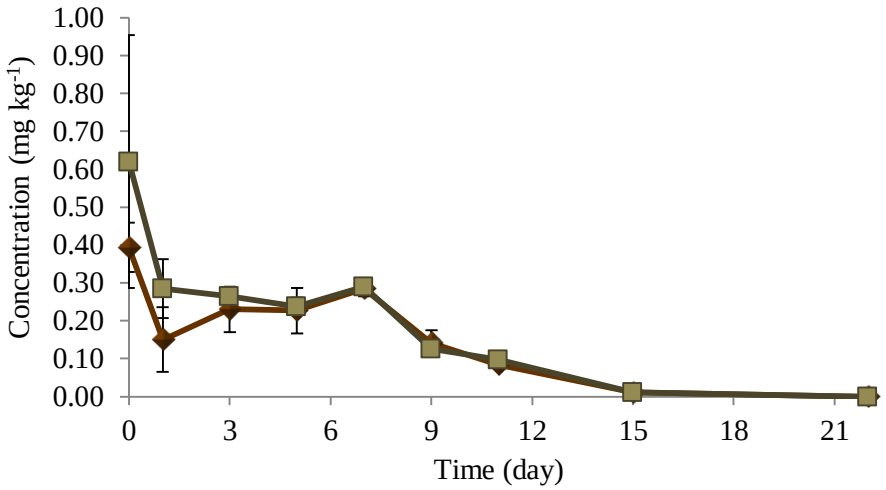


Figure 4.13 The dissipation curves chlorothalonil in cropped topsoil under N.H (◆) and O.F (▩) cultivation systems during wet season. Vertical lines represent standard deviation (n=3).

Chlorothalonil concentration rose slightly on day 3 under N.H condition, but declined continuously in O.F. This trend had also been observed for λ-cyhalothrin during wet season. Heavy downpour on day 2 (20.9 mm) was suspected to washed chlorothalonil residue from green mustard into the topsoil. More intense rainfall in O.F however might have caused some amount of chlorothalonil to leach into deeper layer of soil (Ciglash *et al.*, 2006).

On day 7, chlorothalonil concentration in top soil increased under both cultivation systems. Similar trend was also observed for profenofos and λ-cyhalothrin due to residue wash-off from plant and the 'wick effect' following continuous rainfall at day 5 and day 6. Chlorothalonil residue declined from day 9 onwards, where the terminal residue was no longer detectable on day 22.

During dry season, chlorothalonil residue was still detectable on day 22, while during wet season, the residue has completely dissipated on day 22. Chlorothalonil was reported to dissipate mainly by abiotic processes, volatilisation and biodegradation (Carlos-Rojas *et al.*, 2004). Moreover, the formation of bound residue also frequently correlated with fast dissipation of chlorothalonil in soil (Potter *et al*., 2001). Bound residue formed caused pesticides to be less accessible to degradation, and therefore the residue in present study was still detectable in day 22.

4.5 The degradation of profenofos, λ-cyhalothrin and chlorothalonil in tropical soil under laboratory condition.

The dissipation of pesticides in soil under field condition involved a number of processes (degradation, leaching, volatilisation, surface run-off) which depend on many environmental factors (soil type, climate, and cultivation system) spatial and temporal variability of environmental conditions (Laabs *et al.*, 2002; Ciglash *et al*., 2006). These overlapping processes and factors make further interpretation of dissipation dynamics difficult. By conducting study under standardised laboratory condition, some factors can be isolated therefore easier to understand a specific process which is also contribute to pesticide dissipation in field (Laabs *et al.*, 2002).

Pesticides dissipation rate under field condition was reported to be shorter than under laboratory condition (Laabs *et al.*, 2002; Qin *et al.*, 2006). Faster dissipation under field condition may be due to a number of processes such as surface run-off, leaching, and volatilisation (Ciglash *et al.*, 2006). Variation and fluctuation of soil moisture and temperature in the field may also lead to enhanced microbial and chemical degradation

(Laabs *et al.*, 2002). Besides, sunlight radiation promotes soil surface photolysis, which is absent under laboratory condition (Laabs *et al.*, 2002).

Profenofos, λ-cyhalothrin and chlorothalonil showed an exponential dissipation curve in Semongok soil (Figure 4.14). Initial profenofos, λ-cyhalothrin and chlorothalonil concentration was 5.42 mg kg^{-1}, 4.78 mg kg^{-1} and 4.05 mg kg^{-1}. Lower concentration recovered for λ-cyhalothrin and chlorothalonil was probably due to the formation of non-extractable bound residue in soil which may requires a rigorous acids extraction (Laabs *et al.*, 2000; Potter *et al.*, 2001).

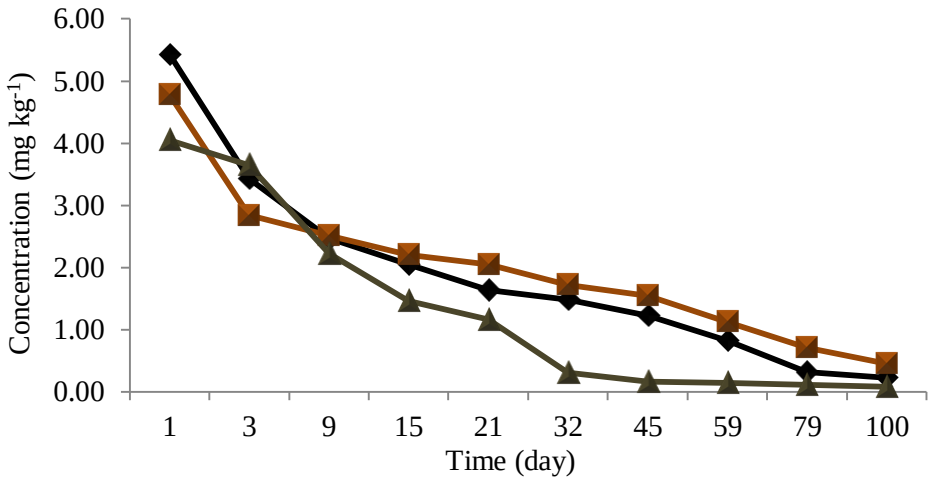


Figure 4.14 Profenofos (◆), λ-cyhalothrin (■) and chlorothalonil (▲) degradation in fresh Semongok field soil incubated under darkness at 25 °C with relative humidity of 80% and soil moisture 30%.

In the first 9 days, about half of their initial concentration have dissipated, with profenofos degraded up to 54% of its initial concentration, λ-cyhalothrin loss was about 48% of its initial concentration, and chlorothalonil recorded 46% loss in concentration. Profenofos and chlorothalonil degraded faster compare to λ-cyhalothrin. Fast initial dissipation of profenofos and chlorothalonil in incubated soil was probably due to volatilisation losses

(Laabs *et al.,* 2002). Pesticides with high vapour pressure have the possibility to volatile under 25 °C. Besides, chlorothalonil and λ-cyhalothrin losses could be attributed to the formation of bound residue (Potter *et al.,* 2001; Carlos-Rojas *et al.,* 2003; He *et al.,* 2008). Bound residue formation may hinder microbial degradation as the pesticides became less accessible to the soil microbes thus impeded degradation (Laabs *et al*., 2002; Malghani *et al.,* 2009).

The degradation rate of these three pesticides however became much slower after the first week. As pesticides started to bind to soil particles, it became less available for microbial degradation. Although mineralisation might still occur, the rate was rather slow due to the formation of bound residues (Potter *et al.,* 2001). Limited microbial community in incubated soil might have also led to a slower dissipation of the three pesticides (Laabs *et al.,* 2002).

On day 32, chlorothalonil exhibited higher rate of dissipation, where 93% of the initial concentration had loss, followed by profenofos with 73% and λ-cyhalothrin, with only 64% loss. λ-cyhalothrin has higher affinity towards soil because of its higher octanol-water coefficient value (K_{ow}) compare to profenofos and chlorothalonil (He *et al.,* 2008). Therefore it is more susceptible to bind with the soil particles and thus it is less available for degradation by the soil microbes (Masutti & Mermut, 2007).

On day 100, chlorothalonil showed the highest loss (98% of the initial concentration) followed by profenofos (96%) and λ-cyhalothrin (91%). More rapid chlorothalonil dissipation was more likely due to the formation of bound residue, while profenofos was reported to be easily degraded in soil compare to λ-cyhalothrin (Potter *et al.*, 2001; Ngan *et*

al., 2005). Although chlorothalonil and λ-cyhalothrin has high affinity to bound to soil, chlorothalonil however exhibited faster dissipation than λ-cyhalothrin.

λ-cyhalothrin dissipation was previously mentioned to be mainly microbial and *via* photodegradation (Hill & Inaba, 1991; He *et al.*, 2008). Its high affinity to soil led to formation of bound residue which hinders microbial degradation to take place (Laabs and Amelung, 2005). Besides, no sunlight has led to slower dissipation of λ-cyhalothrin as photodegradation was inhibited. Slower dissipation was also observed for profenofos and chlorothalonil compare to field experiment which may be partly attributable to the fact that photodegradation was completely inhibited (Qin *et al.*, 2006).

Other than photodegradation, volatilisation rate under field condition is higher compare to laboratory condition. Therefore losses *via* volatilisation in soil under field condition were higher than in incubated soil. Laabs *et al*., (2002) have also reported faster dissipation of several pesticides under field condition compare to incubated soil, where even pesticides with low vapour pressure which does not volatilise under laboratory was reported to volatilise under field condition. Higher dissipation rate under field condition can be correlated with the presence of sunlight and wind which favour photodegradation and volatilisation to occur.

Half-life calculated for profenofos, λ-cyhalothrin and chlorothalonil was 24 days, 37 days, and 15.2 days, respectively. Hypothesis testing suggests that the dissipation of these 3 pesticides in incubated Semongok soil was significantly different (Appendix XX). Variation in the degradation rate of these three pesticides in soil under controlled condition suggested the significant influence of physicochemical properties of individual pesticides

to their degradation rate. The K_{ow} value sequence for the three pesticides are λ-cyhalothrin > chlorothalonil > profenofos and the half-life of the three pesticides in incubated soil was in the sequence of λ-cyhalothrin > profenofos > chlorothalonil.

CHAPTER 5

CONCLUSION

The issue of pest and diseases is a serious challenge to vegetable farmers in Sarawak as it cause severe damage and reduced production yield. Overly dependence on pesticides has led to serious residual problems in crops produced and poses threat to the environment. In order to ensure food safety, MRL has been used as a guideline to control pesticide residue in vegetable production. To avoid MRL violation, farmers need to practise GAP and follow the prescribed pesticides dosage and pre-harvest intervals (PHI). This ensure the produced vegetables are safe to consume.

Pesticides dissipation is a complex process consisting of various mechanisms and is governed by many factors. The dissipation rate of a pesticide would determine its half-life and thus their optimum pre-harvest interval (PHI) in food commodities. Literatures have demonstrated that specific pesticide dissipation pattern varies accordingly to local climate condition which indicates a pesticide half-lives obtained through designed experiments are compound specific and local. Pesticides dissipation in vegetable and soil has been widely studied in temperate and sub-tropical climate. Variation of the results obtained were suggested to be due to climatic factors. Under humid-tropical condition however, the information of pesticides fate and behaviour in crops and soil is still limited and therefore needs more research in order to obtain deeper and better understanding of various kind of pesticides in different types of crops and soil.

Investigation on the dissipation rate of profenofos, λ-cyhalothrin and chlorothalonil in green mustard and soil showed pesticide dissipation depend on the physicochemical

properties of the pesticides itself. Profenofos has higher water solubility and higher vapour pressure than λ-cyhalothrin and chlorothalonil. Therefore it dissipated faster than λ-cyhalothrin and chlorothalonil in both green mustard and soil. In terms of half-life, the sequence was in the order of profenofos < chlorothalonil < λ-cyhalothrin in both green mustard and soil.

Climatic factors such as sunshine, surrounding temperature and rainfall were also proven to affect pesticide's dissipation rate in both green mustard and soil where each pesticides exhibited different dissipation trend during dry and wet season. Profenofos, λ-cyhalothrin and chlorothalonil dissipated faster during dry season, with higher temperature and longer sunshine. In addition, the occurrence of rainfall during dry season had accelerated the dissipation rate of the three pesticides in both green mustard and soil. Although dry season showed faster rate of dissipation (proven with short half-lives obtained), trace amount of residue however was observed to be effectively removed during wet season, where continuous rainfall was suspected to facilitate the residues washed-off from green mustard.

Apart from climatic factors, cultivating systems used (N.H and O.F) also showed significant effect towards the dissipation rate of profenofos, λ-cyhalothrin and chlorothalonil in both green mustard and soil. Although the dissipation of the three pesticides under N.H and O.F showed not much different in terms of half-life, overall results from this study showed that in dry season, faster dissipation took place in O.F while in wet season; faster dissipation took place in N.H. These results suggested the importance of surrounding temperature in accelerating pesticide dissipation rate in both vegetable and soil.

This study has identified that dissipation rate of profenofos, λ-cyhalothrin and chlorothalonil under humid tropical condition were dependence on factors such as physicochemical property, climate condition and cultivation systems. Long pre-harvest interval (PHI) suggested that profenofos, λ-cyhalothrin and chlorothalonil should be applied to green mustard at least 2 weeks prior to harvest in order to ensure the residue have enough time to dissipate below tolerance level during harvest. Whilst in soil, the three pesticides dissipated faster under field condition compare to laboratory condition. This affirmed the involvement of a number of interactive processes have accelerated pesticide dissipation in the environment.

More studies are needed in order to obtain better understanding of other pesticides in different type of vegetables and different application dosage in order to provide vital information for the development of effective guidelines for local farmers in terms of pesticide usage. Besides, pesticides dissipation behaviour in different types of cropped soil needs further research as vegetable cultivation in peat areas of Sarawak is growing from years to years. This is relatively significant to ensure sustainable agriculture is well practiced in Sarawak.

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Appendix I

Profenofos dissipation % in green mustard during dry season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	8.35	0	0	7.14	0.00	0.00
1	4.70	44	44	4.50	37.00	37.00
3	2.57	69	45	3.59	50.00	20.00
5	1.21	86	53	1.46	80.00	59.00
7	0.27	97	78	0.46	94.00	69.00
9	0.07	99	74	0.10	99.00	78.00
11	0.06	99	14	0.01	99.00	90.00
15	0.01	99	83	0.00	100.00	100.00
22	0.00	100	100	0.00	0.00	0.00

Appendix II

Profenofos dissipation in green mustard during wet season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)[a]	Daily dissipation (%)[b]	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	6.60	0.00	0.00	4.72	0.00	0.00
1	4.90	26.00	26.00	4.70	0.42	0.42
3	2.65	60.00	46.00	2.58	45.00	45.00
5	0.41	94.00	85.00	1.41	70.00	45.00
7	0.14	98.00	66.00	0.91	81.00	36.00
9	0.04	99.00	71.00	0.24	95.00	74.00
11	0.03	99.00	25.00	0.05	99.00	79.00
15	0.01	99.00	67.00	0.02	99.00	60.00
22	0.00	100.00	100.00	0.00	100.00	100.00

[a] % of total dissipation = $\frac{Ci-Ct}{Ci} \times 100$

[b] % of daily dissipation = $\frac{Ctx-Cty}{Ctx} \times 100$

Where $Ci = initial\ concentration$

$Ct = concentration\ at\ time\ (day)$

$Ctx = concentration\ at\ time\ x\ (day)$

Cty = concentration at time y (day)

Appendix III

λ-cyhalothrin dissipation in green mustard during dry season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.41	0.00	0.00	0.38	0.00	0.00
1	0.16	61.00	61.00	0.20	47.00	47.00
3	0.06	85.00	63.00	0.04	90.00	80.00
5	0.03	93.00	50.00	0.03	92.00	25.00
7	0.01	98.00	67.00	0.02	95.00	33.00
9	0.03	-93.00	-67.00	0.02	95.00	0.00
11	0.01	98.00	67.00	0.01	97.00	50.00
15	0.01	98.00	0.00	0.01	99.00	50.00
22	0.002	99.00	80.00	0.002	99.00	60.00

Appendix IV

λ-cyhalothrin dissipation in green mustard during wet season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.39	0.00	0.00	0.34	0.00	0.00
1	0.24	39.00	39.00	0.32	5.90	5.90
3	0.15	62.00	38.00	0.12	65.00	63.00
5	0.06	85.00	60.00	0.06	82.00	50.00
7	0.03	92.00	50.00	0.05	85.00	17.00
9	0.01	97.00	67.00	0.04	88.00	20.00
11	0.01	97.00	0.00	0.04	88.00	0.00
15	0.01	97.00	0.00	0.02	94.00	2.00
22	0.00	100.00	100.00	0.00	100.00	100.00

[a] % of total dissipation = $\frac{Ci-Ct}{Ci} \times 100$

[b] % of daily dissipation = $\frac{Ctx-Cty}{Ctx} \times 100$

Where $Ci = initial\ concentration$

$Ct = \ concentration\ at\ time\ (day)$

$Ctx = concentration\ at\ time\ x\ (day)$

Cty = concentration at time y (day)

Appendix V

Chlorothalonil dissipation in green mustard during dry season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	10.03	0.00	0.00	13.22	0.00	0.00
1	9.36	6.70	6.70	7.80	41.00	41.00
3	8.17	19.00	13.00	7.02	47.00	10.00
5	6.72	33.00	18.00	6.32	52.00	10.00
7	5.29	47.00	21.00	5.24	60.00	17.00
9	0.37	96.00	93.00	0.39	97.00	93.00
11	0.47	95.00	-21.00	0.10	99.00	74.00
15	0.14	99.00	70.00	0.08	99.00	20.00
22	0.08	99.00	43.00	0.04	99.00	50.00

Appendix VI

Chlorothalonil dissipation in green mustard during wet season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	11.72	0.00	0.00	11.33	0.00	0.00
1	10.99	6.20	6.20	10.68	5.70	5.70
3	4.79	59.00	56.00	6.44	43.00	40.00
5	3.89	67.00	19.00	2.61	77.00	59.00
7	0.59	95.00	85.00	0.82	93.00	69.00
9	0.44	97.00	25.00	0.79	93.00	3.70
11	0.17	98.00	61.00	0.16	99.00	80.00
15	0.08	99.00	53.00	0.05	99.00	69.00
22	0.02	99.00	75.00	0.01	99.00	80.00

[a] % of total dissipation = $\frac{Ci-Ct}{Ci} \times 100$

[b] % of daily dissipation = $\frac{Ctx-Cty}{Ctx} \times 100$

Where $Ci = initial\ concentration$

$Ct = \ concentration\ at\ time\ (day)$

$Ctx = concentration\ at\ time\ x\ (day)$

Cty = concentration at time y (day)

Appendix VII

Profenofos dissipation in Semongok topsoil during dry season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.0224	0.00	0.00	0.0747	0.00	0.00
1	0.0980	-77.14	-77.14	0.1145	-34.76	-34.76
3	0.0268	16.41	72.65	0.0522	30.39	54.41
5	0.0274	2.24	-2.19	0.0868	-13.94	40.09
7	0.0000	100.00	100.00	0.0106	85.81	87.79
9	0.0000	0.00	0.00	0.0043	94.24	59.43
11	0.0000	0.00	0.00	0.0037	95.05	13.95
15	0.0000	0.00	0.00	0.0074	90.09	-50.00
22	0.0000	0.00	0.00	0.0000	100.00	100.00

Appendix VIII

Profenofos dissipation in Semongok topsoil during wet season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.0330	0.00	0.00	0.0800	0.00	0.00
1	0.0405	-44.36	-44.36	0.0573	28.37	28.37
3	0.0470	-29.78	13.83	0.0760	5.00	-25.00
5	0.0247	25.15	47.45	0.0730	5.00	3.94
7	0.0747	-55.82	-66.93	0.0957	-19.62	23.72
9	0.0396	-20.00	46.99	0.0527	34.13	44.93
11	0.0455	-37.87	-14.90	0.0603	25.00	-12.60
15	0.0244	27.27	47.25	0.0440	50.00	33.00
22	0.0103	68.79	57.79	0.0220	75.00	50.00

[a] % of total dissipation = $\frac{Ci-Ct}{Ci} \times 100$

[b] % of daily dissipation = $\frac{Ctx-Cty}{Ctx} \times 100$

Where $Ci = initial\ concentration$

$Ct = concentration\ at\ time\ (day)$

$Ctx = concentration\ at\ time\ x\ (day)$

Cty = concentration at time y (day)

Appendix IX

λ-cyhalothrin dissipation in Semongok topsoil during dry season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.300	0.00	0.00	0.0233	0.00	0.00
1	0.0267	-77.14	-77.14	0.0300	-22.33	-22.33
3	0.0233	92.23	12.73	0.0264	-13.31	12.00
5	0.0268	91.07	15.02	0.0633	-63.19	-58.29
7	0.0167	94.43	37.69	0.0537	-56.61	15.16
9	0.0016	94.67	90.42	0.0014	94.00	97.39
11	0.0018	99.40	11.11	0.0044	81.11	-68.18
15	0.0000	100.00	100.00	0.0000	100.00	100.00
22	0.0000	0.00	0.00	0.0000	0.00	0.00

Appendix X

λ-cyhalothrin dissipation in Semongok topsoil during wet season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.1367	0.00	0.00	0.0767	0.00	0.00
1	1.0809	-87.35	-87.35	0.0600	21.77	21.77
3	0.0884	35.63	18.51	0.0259	70.88	56.83
5	0.0873	36.35	1.58	0.0687	10.43	-62.30
7	0.0918	32.85	-5.22	0.0690	10.04	-0.44
9	0.0835	38.92	18.37	0.0571	25.68	17.25
11	0.0244	82.15	69.50	0.0332	56.71	44.00
15	0.0109	92.03	55.32	0.0107	86.10	67.78
22	0.0000	100.00	100.00	0.0000	100.00	100.00

[a] % of total dissipation = $\frac{Ci-Ct}{Ci} \times 100$

[b] % of daily dissipation = $\frac{Ctx-Cty}{Ctx} \times 100$

Where $Ci = initial\ concentration$

$Ct = concentration\ at\ time\ (day)$

$Ctx = concentration\ at\ time\ x\ (day)$

Cty = concentration at time y (day)

Appendix XI

Chlorothalonil dissipation in Semongok topsoil during dry season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	1.3000	0.00	0.00	1.8400	0.00	0.00
1	1.8767	-32.04	-32.04	1.5267	17.39	17.39
3	0.3367	74.10	82.06	0.8800	52.17	42.35
5	1.5303	-15.03	-77.97	2.3167	-20.58	-61.90
7	2.0510	-36.58	-25.36	2.5137	-26.80	-7.84
9	1.0720	18.00	47.80	1.1506	37.50	54.18
11	0.9367	27.95	14.26	0.0480	97.40	95.82
15	0.8233	36.70	12.10	0.0643	96.51	-25.35
22	0.4233	67.44	48.59	0.0812	95.59	20.81

Appendix XII

Chlorothalonil dissipation in Semongok topsoil during wet season.

Day	N.H concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)	O.F concentration (mg kg^{-1})	Total dissipation (%)	Daily dissipation (%)
0	0.3933	0.00	0.00	0.6201	0.00	0.00
1	0.1504	61.76	61.76	0.2850	54.03	54.03
3	0.2307	41.34	-53.40	0.2638	57.46	7.44
5	0.2266	42.38	1.78	0.2378	61.65	9.86
7	0.2851	27.51	-25.82	0.2907	53.13	-22.25
9	0.1409	64.18	50.57	0.1261	79.67	56.62
11	0.0831	78.87	41.02	0.0974	84.30	22.76
15	0.0109	97.22	86.88	0.0107	98.28	89.01
22	0.0000	100.00	100.00	100.00	100.00	100.00

[a] % of total dissipation = $\frac{Ci-Ct}{Ci} \times 100$

[b] % of daily dissipation = $\frac{Ctx-Cty}{Ctx} \times 100$

Where $Ci = initial\ concentration$

$Ct = concentration\ at\ time\ (day)$

$Ctx = concentration\ at\ time\ x\ (day)$

Cty = concentration at time y (day)

Appendix XIII

Statistic testing on the amount of rainfall during dry and wet season.

Anova: Two-Factor With Replication

SUMMARY	Sunshine	Temperature	Rainfall	Total
Dry season				
Count	22	22	22	66
Sum	100.8	719.2	304.1	1124.1
Average	4.58182	32.6909	13.8227	17.0318
Variance	11.2092	4.12372	512.849	309.585
Wet season				
Count	22	22	22	66
Sum	62.9	708.6	276.4	1047.9
Average	2.85909	32.2091	12.5636	15.8773
Variance	3.25515	2.68848	239.119	230.528
Total				
Count	44	44	44	
Sum	163.7	1427.8	580.5	
Average	3.72045	32.45	13.1932	
Variance	7.82318	3.38628	367.646	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	43.9882	1	43.9882	0.34133	0.56011	3.91632
Columns	18860.5	2	9430.26	73.1742	8.1E-22	3.0681
Interaction	8.64955	2	4.32477	0.03356	0.96701	3.0681
Within	16238.1	126	128.874			
Total	35151.3	131				

RESULTS:

There is no significant difference of the amount of sunshine, temperature and rainfall on each day of sampling.

There is no significant difference of the amount of sunshine, temperature and rainfall during dry and wet season.

Appendix XIV

Profenofos: Growing season and cultivating systems effect onto profenofos residue on green mustard.

Anova: Two-Factor With Replication

SUMMARY	Net house	Open field	Total
DRY SEASON			
Count	9	9	18
Sum	17.24	17.26	34.5
Average	1.9155555	1.91777777	1.91666
Variance	8.3673527	6.67126944	7.077
	8	4	
wet season			
Count	9	9	18
Sum	14.78	14.63	29.41
Average	1.6422222	1.62555555	1.63388
Variance	2	6	9
	6.3138444	3.76495277	4.74303
	4	8	7
Total			
Count	18	18	
Sum	32.02	31.89	
Average	1.7788888	1.77166666	
Variance	9	7	
	6.9285751	4.93376764	
	6	7	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.7196694	1	0.71966	0.11460	0.73716	4.14909
	4		9	9	8	7
Columns	0.0004694	1	0.00046	7.48E-05	0.99315	4.14909
	4		9		5	7
Interaction	0.0008027	1	0.00080	0.00012	0.99104	4.14909
	8		3	8	9	7
Within	200.93935	32	6.27935			
	6		5			

Appendix XV

λ-cyhalothrin: Growing season and cultivating systems effect onto λ-cyhalothrin residue on green mustard.

Anova: Two-Factor With Replication

SUMMARY	Net house	Open field	Total
DRY SEASON			
Count	9	9	18
Sum	2.801	2.829	5.63
	0.3112222	0.31433333	0.31277
Average	2	3	8
	0.2905351	0.25745761	0.25788
Variance	1	1	1
wet season			
Count	9	9	18
Sum	0.9	0.99	1.89
Average	0.1	0.11	0.105
			0.01650
Variance	0.018375	0.01665	9
Total			
Count	18	18	
Sum	3.701	3.819	
	0.2056111	0.21216666	
Average	1	7	
	0.1571792	0.14004384	
Variance	7	6	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	0.3885444		0.38854	2.66574	0.11233	4.14909
Sample	4	1	4	7	3	7
	0.0003867		0.00038	0.00265	0.95923	4.14909
Columns	8	1	7	4	7	7
	0.0001067		0.00010	0.00073	0.97857	4.14909
Interaction	8	1	7	3	5	7
	4.6641417		0.14575			
Within	8	32	4			
	5.0531797					
Total	8	35				

Appendix XVI

Chlorothalonil: Growing season and cultivating systems effect onto chlorothalonil residue on green mustard.

Anova: Two-Factor With Replication

SUMMARY	Net house	Open field	Total
DRY SEASON			
Count	9	9	18
Sum	40.633333	40.2133333	80.8466
Average	4.5148148	4.46814814	4.49148
Variance	18.143780	21.6011919	18.7040
	9	8	9
wet season			
Count	9	9	18
Sum	32.69	32.89	65.58
Average	3.6322222	3.65444444	3.64333
Variance	2	4	3
	22.256844	21.4808777	20.5825
	4	8	9
Total			
Count	18	18	
Sum	73.323333	73.1033333	
Average	3	3	
Variance	4.0735185	4.06129629	
	2	6	
	19.218256	20.4491805	
	8	7	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.4741975	1	6.47419	0.31020	0.58142	4.14909
	3		8	5	9	7
Columns	0.0013444	1	0.00134	6.44E-05	0.99364	4.14909
	4		4		6	7
Interaction	0.0106777	1	0.01067	0.00051	0.98209	4.14909
	8		8	2	5	7
Within	667.86156	32	20.8706			
			7			
Total	674.34778	35				

Appendix XVII

Statistical testing on the effect of types of pesticide applied and cultivating systems used onto pesticide residue concentration on green mustard during dry season.

H01: There is no significant difference between mean of type of pesticides with the residue concentration

H02: There is no significant difference between the cultivating systems with the residue concentration

H03: There is no interaction between type of pesticides used and cultivating system with the residue concentration

Anova: Two-Factor With Replication

SUMMARY	Net house	Open field	Total
Profenofos			
Count	9	9	18
Sum	17.24	17.26	34.5
Average	1.91555 6	1.91777 8	1.91666 7
Variance	8.36735 3	6.67126 9	7.077
λ-cyhalothrin			
Count	9	9	18
Sum	2.801	2.829	5.63
Average	0.31122 2	0.31433 3	0.31277 8
Variance	0.29053 5	0.25745 8	0.25788 1
Chlorothalonil			
Count	9	9	18
Sum	40.6333 3	40.2133 3	80.8466 7
Average	4.51481 5	4.46814 8	4.49148 1
Variance	18.1437 8	21.6011 9	18.7040 9
Total			
Count	27	27	
Sum	60.6743 3	60.3023 3	

Average	2.247198	2.23342
Variance	11.36208	11.81647

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	159.9822	2	79.99109	8.674006	0.000608	3.190727
Columns	0.002563	1	0.002563	0.000278	0.986769	4.042652
Interaction	0.007303	2	0.003652	0.000396	0.999604	3.190727
Within	442.6527	48	9.221931			
Total	602.6447	53				

RESULT

H01: There is no significant difference between mean of type of pesticides with the residue concentration. Rejected, p-value < 0.05
Therefore, types of pesticides have significant effect on the residue concentration.
This is because different formulation ised has different active ingredients (a.i) percentage.

H02: There is no significant difference between the cultivating systems with the residue concentration. Accepted, p-value>0.05
Cultivating systems used showed no significant effect to the residue concentration of pesticides.
This could be due to a very small difference in terms of concentration which has cused it to be statistically indifferent.

H03: There is no interaction between type of pesticides used and cultivating system with the residue concentration. Accepted, p-value>0.05
Types of pesticides and cultivating system used have no significanct effect to the residue concentration detected.

Appendix XVIII

Statistical testing on the effect of types of pesticide applied and cultivating systems used onto pesticide residue concentration in green mustard during wet season.

Anova: Two-Factor With Replication

SUMMARY	Net house	Open field	Total
Profenofos			
Count	9	9	18
Sum	14.78	14.63	29.41
Average	1.64222222	1.625556	1.633889
Variance	6.31384444	3.764953	4.743037
λ-cyhalothrin			
Count	9	9	18
Sum	0.9	0.99	1.89
Average	0.1	0.11	0.105
Variance	0.018375	0.01665	0.016509
Chlorothalonil			
Count	9	9	18
Sum	32.69	32.89	65.58
Average	3.63222222	3.654444	3.643333
Variance	22.2568444	21.48088	20.58259
Total			
Count	27	27	
Sum	48.37	48.51	
Average	1.79148148	1.796667	
Variance	10.9676131	9.962654	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	113.371026	2	56.68551	6.315753	0.003673	3.190727
Columns	0.00036296	1	0.000363	4.04E-05	0.994952	4.042652
Interaction	0.00355926	2	0.00178	0.000198	0.999802	3.190727
Within	430.812356	48	8.975257			
Total	544.187304	53				

RESULT

H01: There is no significant difference between mean of type of pesticides with the residue concentration. Rejected, p-value < 0.05.
Therefore, types of pesticides have significant effect on the residue concentration.
This is because different formulation ised has different active ingredients (a.i) percentage.

H02: There is no significant difference between the cultivating systems with the residue concentration. Accepted, p-value>0.05.
Cultivating systems used showed no significant effect to the residue concentration of pesticides.
This could be due to a very small difference in terms of concentration which has cused it to be statistically indifferent.

H03: There is no interaction between type of pesticides used and cultivating system with the residue concentration. Accepted, p-value>0.05.
Types of pesticides and cultivating system used have no significanct effect to the residue concentration detected.

Appendix XIX

Half-life variation of profenofos, λ-cyhalothrin and chlorothalonil on green mustard during dry and wet season.

Anova: Two-Factor Without Replication (Net house)

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Profenofos	2	2	1	0.1568
λ-cyhalothrin	2	3.69	1.845	0.88445
Chlorothaloni l	2	3.24	1.62	0.6962
Dry season	3	2.93	0.976667	0.055033333
Wet season	3	6	2	0.4113

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.766033 3	2	0.383017	4.597119424	0.17866 3	19
Columns	1.570816 7	1	1.570817	18.85357071	0.04916 2	18.5128 2
Error	0.166633 3	2	0.083317			
Total	2.503483 3	5				

Anova: Two-Factor Without Replication (Open field)

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Profenofos	2	2.42	1.21	0.605
λ-cyhalothrin	2	4.94	2.47	2.9768
Chlorothalonil	2	2.88	1.44	0.6728
Dry season	3	2.77	0.923333	0.090033
Wet season	3	7.47	2.49	1.0969

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Rows	1.80093333	2	0.900467	3.143356	0.2413503	19
Columns	3.68166667	1	3.681667	12.85199	0.0697646	18.51282
Error	0.57293333	2	0.286467			
Total	6.05553333	5				

Dry and wet season has no significant effect onto pesticide's half-life

Different type of pesticides showed significant difference in their half-lives on green mustard.

Appendix XX

Hypothesis testing of pesticides lab incubation study

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Lab Incubation	3	14.25	4.75	0.472044
	3	9.923333	3.3077778	0.173881
	3	7.203333	2.4011111	0.02507
	3	5.72	1.9066667	0.155511
	3	4.843333	1.6144444	0.198293
	3	3.516667	1.1722222	0.57197
	3	2.923333	0.9744444	0.523626
	3	2.083333	0.6944444	0.252559
	3	1.14	0.38	0.0927
	3	0.7567	0.2522333	0.034714
Profenofos	10	19.08337	1.9083367	2.473907
λ-cyhalothrin	10	19.93667	1.9936667	1.5395
Chlorothalonil	10	13.34	1.334	2.277204

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	54.1891	9	6.0210118	44.6664	1.7E-10	2.45628
Columns	2.57435	2	1.2871732	9.548792	0.00149	3.55456
Error	2.42639	18	0.1347996			
Total	59.1898	29				

Appendix XXI

Recorded field temperature and humidity during sampling in wet season.

Season/sampling day	Time	Weather	Temperature (°C)	Humidity (%)
Wet season Day 0	12.00 p.m	Sunny	33.6	64
Day 1	9.30 a.m	Cloudy	31.4	68
Day 3	9.00 a.m	Sunny	33.2	66
Day 5	11.30 a.m	Sunny	33.6	61
Day 7	9.00 a.m	Cloudy	31.5	66
Day 9	11.00 a.m	Sunny	33.4	62
Day 11	11 a.m	Cloudy	32.7	64
Day 15	9.30 a.m	Cloudy	30.4	63
Day 21	9.30 a.m	Cloudy	28.4	72

Appendix XXII

Recorded field temperature and humidity during sampling in dry season.

Season/sampling day	Time	Weather	Temperature (°C)	Humidity (%)
Wet season				
Day 0	1.00 p.m	Sunny	34.8	64
Day 1	9.30 a.m	Cloudy	30.0	68
Day 3	12.00 p.m	Sunny	36.2	61
Day 5	12.30 p.m	Sunny	33.5	61
Day 7	9.00 a.m	Sunny	33.4	63
Day 9	11.00 a.m	Sunny	33.6	61
Day 11	9.00 a.m	Sunny	35	60
Day 15	9.00 a.m	Cloudy	30.2	67
Day 21	9.30 a.m	Cloudy	30.1	70