

Dissipation and Persistence of Carbofuran and its Secondary Metabolite, 3-Ketocarbofuran in Brinjal (*Solanum melongena*) and Soil under Humid Tropical Climate

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Dissipation and Persistence of Carbofuran and its Secondary Metabolite, 3-Ketocarbofuran in Brinjal (*Solanum melongena*) and Soil under Humid Tropical Climate

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Carbofuran (2,3-dihydro-2,2-dimethyl-benzofuranyl-N-methylcarbamate) is a broadspectrum insecticide-nematicide and appeared to be the most effective insecticide in controlling pests. Although it is banned in many countries in the world, it is widely used in Asia, Australia and South America. Carbofuran is a legally registered pesticide and commonly used in vegetable farming in Malaysia due to reasonable price in the market. In this study, the persistence and degradation of carbofuran were examined under humid tropic field and laboratory conditions. A total of 162 brinjal (Solanum melongena) plants were grown at Agricultural Research Centre, Semongok, Sarawak and carbofuran was applied when plants began to bear fruits. Prior to carbofuran residue analysis, the physicochemical properties of crop soil were studied. Modified QuEChERS method was used to determine the residue of carbofuran in soil and brinjal fruits and leaves. Meanwhile, in this study, the laboratory degradation of carbofuran under the effect of pH, moisture and temperature were studied using response surface methodology (RSM). A total of 24 experiments were carried out by incubating sets of sterilised soil for 24 hours according to inscribe central composite design (CCD). The percentage of carbofuran loss was calculated and fitted according to linear, interaction and quadratic models whereby its quality is measured on root mean square error (RSME) and regression coefficients, R². Under field condition, in order to comply with the maximum residue limit (MRL) of 0.01 mg/kg, the pre-harvest interval suggested for brinjal was 28 days. The average half-life of carbofuran in soil, brinjal leaves and fruits are 1.24, 3.22 and 10.33 days, respectively. On the other hand, under laboratory condition, it was experimentally verified that linear model has the lowest RSME with an error of 5.82% and it is best describes the degradation of carbofuran. All three factors (pH, moisture and temperature) were found to correlate positively to the loss of carbofuran in soil. The soil pH

establishes significant effect (p < 0.05) on the degradation which indicates chemical hydrolysis as the major pathway in this study.

Keywords: Carbofuran, dissipation, persistence, response surface methodology, *Solanum melongena*

Disipasi serta Kesinambungan Karbofuran dan Metabolitnya, 3-Ketocarbofuran di dalam Terung (<u>Solanum melongena</u>) dan Tanah bagi Kawasan Klimat Tropikal Lembap

ABSTRAK

Karbofuran (2,3-dihidro-2,2-dimetil-benzofuranil-N-metilkarbamat) adalah sejenis racun perosak spektrum luas dan ianya yang amat berkesan dalam menangani serangga perosak pada tanaman. Walaupun penggunaannya telah diberhentikan di beberapa negara, racun perosak karbofuran masih dipraktikkan secara luas di negara-negara Asia, Australia dan Amerika Selatan. Karbofuran dikenali dengan harganya yang murah malah disebabkan ianya juga telah didaftarkan secara sah, penggunaannya di Malaysia diperluaskan lagi demi mencegah masalah serangga perosak. Kajian tentang kesinambungan dan degradasi karbofuran telah dibahagikan kepada dua bahagian iaitu pada kondisi tanaman ladang terbuka dan kajian di dalam makmal. Sebanyak 162 tanaman terung (Solanum melongena) ditanam di Pusat Penyelidikan Pertanian, Semongok, Sarawak dan sebaik sahaja tanaman tersebut mula berbuah, racun perosak karbofuran disembur ke atas tanaman. Sifat fizikokimia tanah telah dikaji sebelum analisis residu karbofuran dijalankan. Kaedah QuEChERS telah digunakan untuk menentukan residu karbofuran dalam tanah, buah dan daun terung. Di samping itu, kesan faktor pH, kelembapan dan suhu terhadap degradasi karbofuran telah dikaji menggunakan metadologi permukaan tindak balas (RSM). Sejumlah 24 set eksperimen telah dijalankan menggunakan kaedah pusat reka bentuk komposit (CCD) dengan mengeram tanah yang steril selama 24 jam. Peratusan residu karbofuran telah dipadankan kepada model linear, interaksi dan kuadratik manakala sifat kualiti model tersebut dilihat dari min ralat kuasa dua (RSME) dan koefisien regresi (R^2). Bagi bahagian pertama kajian, profil residu karbofuran menunjukkan peningkatan pada maksimum seterusnya menurun secara beransur-ansur sehingga ia mencapai had residu maksimum (MRL) kurang dari 0.01 mg/kg. Oleh itu, kajian ini telah menetapkan 28 hari sebagai jarak waktu yang selamat bagi pemetikan terung dengan MRL 0.01 mg/kg. Tambahan pula, dalam kajian pertama ini, purata separuh hayat bagi karbofuran di dalam tanah, daun dan buah terung masing-masing adalah 1.24, 3.22 dan 10.33 hari. Manakala, bagi kajian di dalam makmal, kajian ini telah mendapati model linear sebagai model yang sesuai untuk menerangkan degradasi karbofuran disebabkan RSME yang terendah, dengan ralat 5.82% berbanding model interaksi dan kuadratik. Ketiga-tiga faktor (pH, kelembapan dan suhu) berhubung secara positif terhadap peratusan residu karbofuran. Menurut hasil kajian yang didapati, faktor pH telah memberi kesan yang lebih signifikan (p < 0.05) dan mencadangkan bahawa hidrolisis sebagai kaedah metabolisme utama bagi degradasi karbofuran.

Kata kunci: Disipasi, karbofuran, kesinambungan, permukaan tindak balas, Solanum melongena

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LIST OF ABBREVIATIONS

ACHE	Acetylcholinesterase
ACH	Acetylcholine
Ca	Calcium
CCD	Central Composite Design
CEC	Cation Exchangeable Capacity
CO ₂	Carbon dioxide
DOA	Department of Agriculture
FAO	Food and Agriculture Organization
GAP	Good Agriculture Practice
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
H ₂ O	Water
HNO ₃	Nitric acid
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
ISS	Integrated Sensor Suit
Κ	Potassium
LOD	Limit of Detection
Mg	Magnesium
MRL	Maximum residual limit
N ₂	Nitrogen gas
Na	Sodium
NaOH	Sodium hydroxide
ND	Non-detectable
PHI	Pre-harvest interval

QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe
rcf	relative centrifugal force
RSD	relative standard deviation
RSM	Response surface methodology
RSME	Root mean square error
SANCO	South African National Civic Organization
t _{1/2}	Half-life
UNIMAS	Universiti Malaysia Sarawak
USA	United States of America
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Research Background

Vegetables production is a profitable business for local farmers, whereby demand is increasing every day. As population grows, demand for food increased steadily which have led to greater demand for fresh vegetables (Sheng et al., 2008). In Sarawak, from the year 2012 to 2017, the area for plantation of vegetables has increased from 4,230 to 5,031 hectares for vegetables and 3,073 to 4,533 hectares for cash crops (DOA Malaysia, 2012, 2017). There are three main categories of vegetables: leafy vegetables (e.g. Chinese kale, Chinese spinach and leaf mustard), fruit vegetables (e.g. eggplant, okra, and bitter gourd) and cash crops (e.g. maize, sweet potato and sugar cane) (DOA Malaysia, 2012).

Eggplant or brinjal (*Solanum melongena*) is a member of the Solanaciae family which also includes tomato, pepper and potato (Gopalakrisnan, 2007). It is one of the most important vegetables in the South-East Asia (Srinivasan, 2008). Brinjal is widely planted fruit vegetables in Sarawak with the planting area of 184 hectares, giving the production output of 2,316 metric tonnes in 2017 (Figure 1.1). Other commonly planted vegetables are cucumber, long bean and okra. Brinjals have short crops cycle; they can be harvested within six to eight weeks after planting. It does not have a long storage life, thus it should be handled carefully to avoid bruising as pitting and decay will occur during storage (Daunay, 2008).



Figure 1.1: Estimated area of fruit vegetables planted in Sarawak in 2016 and 2017 (DOA Malaysia, 2016, 2017)

Demands for fresh vegetables with no apparent bruises, discolouration and holes attributed to pest infestation and plant diseases have exerted substantial pressure on farmers to produce good quality vegetables. Therefore, agrochemicals such as pesticides and fertilizers are widely used to produce high quality vegetables in a large quantity (Juraske et al., 2009).

Pesticides are mainly used to control pests on crops and in soils. In the tropics, pesticides are applied to vegetable crops throughout their growing seasons and on soils after harvest (Chai et al., 2014). Pesticides are also used before or after harvest to protect commodity from deterioration during storage, transport, distribution and processing (Chai et al., 2012).

Carbofuran is one of the common pesticides used in Malaysia. It is a broad spectrum systemic acaricide, insecticide and nematicide, included in the general group of carbamate derivative pesticide. As stated in many research papers, carbofuran is widely used for the

control of soil dwelling and foliar feeding insects (Benitez et al., 2002). The degradation and metabolism of carbofuran is rapid (approximately 20 minutes) which occurs with the organisms and in plants, soil and water (Otieno et al., 2010). There are few degradation pathways of carbofuran such as hydrolysis, photolysis (Achik & Schiavon, 1989), redox reaction (Hodgson et al., 1991) and also through microbial activity (Lalah et al., 2001). Although it is credited with success in increasing farm yield and ensuring quality crops, its potential to harm human and environment has received greater attentions. Carbofuran has proven to be toxic and could affect the aquatic environment, providing a potential route of exposure to fish, mammals and birds (Fishel, 2014).

Therefore, in order to appease agricultural and environmental interests, a better understanding of the fate of carbofuran in both environment and in plant is needed. By conducting field and laboratory studied of carbofuran, the fate and dissipation rate of the residual behaviour in the environment and treated crops in terms of half-life and also optimum pre-harvest interval can be determined.

1.2 Problem Statement

Although there are benefit uses of pesticides, there have also been many problems associated with their use. When pesticides are used, they do not usually persist in the location where they are applied. Pesticides are mobile in the environment and often move through water, air and soil (Fishel, 2014). This could cause harm when they are in contact with other non-target organisms and thus drastically alter the natural balance of the ecosystem. Pesticide residues can be present in a great variety of daily foods and beverages. The residues in most situations do not meet the Maximum Residue Limit (MRL). Those who ignores the significance of MRL may underestimate the actual health risk, as in the case of simultaneous exposure to two or more chemical substance and may have stimulatory effects (Fanke & Juraske, 2013).

Carbofuran is one of the most toxic carbamate pesticides. Its toxicity has caused millions of bird deaths in 1991 (Erwin, 1991). Therefore, it is banned in certain parts in the U.S and Kenya. However, carbofuran is broadly used in Malaysia especially on crops such as brinjals, okras, watermelons, paddy fields and in oil palm plantations. Although it is widely used in Malaysia, there are limited studies on uptake and degradation behaviour of carbofuran in tropical soil environment (Farahani et al., 2007). Therefore, there is a need to conduct research on the fate and persistence of carbofuran to understand its behaviour under tropical conditions and whether the crops applied with carbofuran is able to meet the Maximum Residual Limit (MRL) according to Malaysia's Food Regulations 1985.

1.3 Objectives

The objectives of this study are:

- i. To determine the uptake of carbofuran and its metabolites in brinjals;
- ii. To determine the dissipation rate, half-life $(t_{1/2})$ and pre-harvest intervals of carbofuran in brinjal and soil under open field condition;
- iii. To determine the optimum condition for degradation of carbofuran in soil using response surface methodology (RSM)

1.4 Chapter Summary

The thesis is organized into five chapters. Chapter 1 begins with an introduction to the background of the study. It then describes the problems faced regarding this study and the specific research objectives.

Chapter 2 presents a review of the literature that connects the types and usage of pesticides and its dissipation mechanisms in soil. It is then broken down specifically to carbofuran, an insecticide that was used in this study to determine its behaviour in soil and vegetables.

Chapter 3 describes the research design and methodology used to investigate the research hypotheses. It outlines the study's field and experimental design, soil analysis, and data collection process and data analysis approach.

Chapter 4 reports the results of the study. It discusses the method validation, recovery of carbofuran and the half-life of carbofuran under field and laboratory settings. This chapter also presents the equation modelling to test the hypotheses of this study by using RSM.

Chapter 5 concludes the thesis by summarizing the research findings and provides recommendations to further apprehend the behaviour and proper usage of carbofuran in Malaysia on crops.

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CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides

Chemical pesticides have been a boon for developing nations to eliminate insect-borne, endemic ailments, to produce sufficient food, and to preserve or restore forests, plantations and fibre (Ecobichon, 2001). Pesticides are nowhere more valuable than in developing countries particularly those in tropical regions seeking to engage in the global economy by providing temperate climates countries with fresh fruits and off-seasonal vegetables. Such developing nations become essential 'breadbasket' for the world, being able to produce two or even three crops annually. Nevertheless, such goals cannot be accomplished without the increased use of pesticides primarily insecticides, herbicides and fungicides, which are not commonly used in conventional farming practices (Matthews, 2008). According to the Food and Agriculture Organization of United Nations (FAO) (1986), pesticides can be defined as any substance or mixture of substances intended for preventing, destroying or controlling any pest including vectors of human or animal diseases and unwanted species of plants or animals. Pesticides are usually used either before or after harvest in order to protect the commodity from degeneration during storage, transport, distribution and processing of food, agricultural commodities or animal feeds (Stephenson et al., 2006).

2.2 Types of pesticides

There are two modes of entry of pesticides; systemic and non-systemic contact pesticides. Systemic contact pesticides are chemical compounds that absorbed by a plant or animals when applied to untreated tissues, soil, seeds, or leaves. The chemicals will circulate

through the tissues of the plant, eradicate the insects which feed on them (Raju & Naik, 2006). They are effective at killing weeds even when with partial spray coverage of the pesticides. Systemic contact pesticides permeated the tissues of the plant affectively, and pass through the plant vascular system to eliminate target pests. Furthermore, few pesticides are considered to be locally systemic and only pass to a distance that is not far off the point of contact. Such pesticides are 2,4-dichlorophenoxyacetic acid (2, 4-D) and glyphosate insecticides (Salman & Hameed, 2010).

Contact or non-systemic pesticides are topical pesticides that can be washed off of a plant easily before consumption. They produce the desired effect when they come in contact with the pest. To activate it, non-systemic pesticides must come into physical contact with the pest. Pesticide reaches the pest's body via their epidermis while in contact which lead to death by poisoning. The plant does not necessarily absorb or uptake contact pesticide through its leaves; it just persist on the plant's exterior. Contact pesticides can easily be flushed or drained from root region of the plant before harvesting, leaving little to no residue behind (Kubik et al., 2000). Common examples of non-systemic pesticides are fumigants or repellents. Fumigants are pesticides that act or get rid of the target pests through vapour. Most of their active ingredients are in liquid form when stored under high pressure and convert to gas form upon releasing. Fumigants are used to extract pest from crops and soil. Repellents however is used to repel the pest and not exactly kill, but they are repugnant enough to keep pests away from treated areas (Ducom, 2006). Repellents are majorly used in public health application to impede mosquitoes and bloodsucking insects (ticks and black flies) from livestock, pets or humans (Debboun & Strickman, 2013).

Pesticides can be classified in many different ways according to the target pest, the compound structures and the degree of hazard involved (WHO, 1990). The word "pesticide" is

a broad term that includes insecticides (used to control insects and pests), fungicides (used to control fungal infection in plants), herbicides (used to control weeds), nematicides (used to control nematodes) and rodenticides (to control rodents) (Zacharia, 2011). Pesticides are also categorized as either a chemical pesticide or biopesticides depending to its sources of origin. Biological pesticides or known as biopesticides are host specific in the sense that it acts on the target species and strongly associated organisms. It is a group of pesticides extracted from animals, plants and microorganisms (bacteria, viruses, fungi and nematods). That will include microbial pesticides, embedded protectants in plant and biochemical pesticides (Regnault-Roger et al., 2005). Biopesticides are environmentally friendly because they are less toxic, easily decomposed and only required in small quantities (Gilden et al., 2010). Moreover, biopesticides are less susceptible to genetic modification in plant populations which makes it an advantage to crops production. This reflects the low probability of resistance to pesticides which is hardly found in chemical pesticides.

Meanwhile, chemical pesticides are non-specific on large group of non-target organisms with a wide range of activities. Chemical pesticides are hazardous as it cause some pollutants to the ecosystem due to its non-biodegradable properties and poisonous to the environment (Dutta, 2015). In agricultural sectors, chemical pesticides or insecticides and fungicides are the most widely used in vegetables production. It can be further subdivided into carbamate, organophosphorus, organochlorine, pyrethroid, organic, inorganic and fumigant (Pimentel, 2009). Carbamate, organophosphorus and pyrethroids compounds are the most commonly used pesticides in developing countries (WHO, 1990).

2.3 Carbamate Insecticides

Carbamates (CM) are a wide class of synthetic compounds which are used for a number of purposes, such as insecticides, agents of warfare and therapeutic medicine for veterinary and human. These compounds are extensively used to control vectors such as mosquitoes, ticks and also to protect public health from diseases such as malaria, Lyme diseases and others (Wang et al., 2013). Currently, CMs (hereinafter known as anticholinesterases) are the most commonly used pesticides in agriculture and forestry around the world. This occurrence is relatively due to their absence of residue persistence in the environment and in mammalian species, as well as to the emergence of lesser persistence as compared with organochlorine pesticides (Mnif et al., 2011). Nevertheless, anticholinesterase pesticides are extremely toxic due to its lack species selectivity thus cause severe threat to public health and environment (Nicolopoulou-Stamati et al., 2016).

Carbamates are synthetic organic pesticides used on a large scale worldwide (Paiga et al., 2009). They are used as insecticides, fungicides, nematocides, acaricides, molluscicides, sprout inhibitors or herbicides. Carbamate insecticides are derivatives of carbamic acid, NH₂COOH with general formula as shown in Figure 2.1 where R is an alcohol, oxime or phenol and R^1 is hydrogen or a methyl group (Machemer & Pickel, 1994).



Figure 2.1: General structure of carbamate (Wayland & Laws, 1991)

Carbamates act similarly as organophospate although they differ chemically. Organophosphates are derivatives of phosphoric acid while carbamates are derived from carbamic acid. The theory behind the use of carbamate pesticides is equivalent to that of organophsphate pesticides since it affects the transmission of nerve signals that lead to the death of the pest by poisoning. When applied to crops or directly to the soil, carbamates usually persist from a few hours to several months (Yadev et al., 2015). They are commonly applied as surface sprays or as baits for household pests (Goad et al., 2004). They are also often used as stomach and contact posion as well as fumigant. This pesticide can be quickly degraded naturally with minimal environmental pollution. A few examples of carbamates are carbofuran, aldicarb, carbaryl, propoxur, oxamyl and terbucarb (Zacharia, 2011).

2.3.1 Carbofuran

Carbofuran (2,3-dihydro-2,2-dimethyl-benzofuranyl-N-methylcarbamate) (Figure 2.2) is a broad-spectrum systemic insecticide, acaricide and nematicide. It is marketed under the trade name of Furadan by FMC Corporation and Curatter which contains active ingredient of carbofuran. It is either applied as granular formulations or foliar spray (Kale et al., 2001). According to Evert (2002), carbofuran insecticide is usually formulated as flowable or wettable powder where pure carbofuran is present as odourless to mildly aromatic white crystalline solid.

Carbofuran is used to control soil-dwelling and foliar-feeding insects such as corn rootworms, wireworms, boll weevils, mosquitoes, alfalfa weevil, aphids and white grubs (Trotter et al., 1991). It is considered to be more persistent than other carbamate or organophosphate insecticides. Carbofuran is commonly applied on sugarcane, sugar beet, maize, rice and coffee (Otieno et al., 2010). According to Rajagopal et al. (1984), carbofuran is widely used in rice to prevent and eliminate pests such as brown plant hopper (*Nilaparvata* *lugensi*), green leaf hopper (*Nephotettix virescens*), stem borers (*Tryphorhiza incertules*) and the whorl maggot (*Hydrellia philippina*) which causes widespread damage to rice. Carbofuran is also effective in controlling certain pests that are resistant to organophophate pesticides such as white flies, leafminers, bees, mealy bugs, scale insects, cockroaches, wasps and aphids. In Malaysia, carbofuran is widely used in paddy fields and in oil palm plantation for the control of rodents and rhinoceros beetle, respectively (Farahani et al., 2007).



Figure 2.2: Chemical structure of carbofuran (Kawamoto & Makihata, 2003)

2.3.2 Toxicity of Carbofuran

Carbofuran is most widely used in agriculture and forestry as insecticide, nematicide and acaricide. Its widespread use contaminates the air, animas, surface water and groundwater with carbofuran and its metabolites which may affect the health of everyone (Zuo et al., 2005). Carbofuran shows moderate mammalian toxicity but is very toxic to invertebrates and birds (Otieno et al., 2011). In 1991, carbofuran was banned due to its toxicity in granular forms to bird. It seems that the granular forms are mistaken as foods by the birds (Erwin, 1991). Field studies with silica-based carbofuran granules have shown high mortalities in birds and result in numerous reports of bird kills (Mineau & Service, 1993). Due to its availability and low price, carbofuran has also been misused as an acaricide for control of tick and small mammals such as moles. In Kenya, carbofuran poisoning was reported in birds, hyenas, camels, lions and hippos since 2003, involving indirect and direct pathways (Oteino et al., 2010a). Carbofuran has a relatively high mammalian toxicity (oral LD50 8-11 mg/kg in rats) and highly hazardous to invertebrates and birds thus it should be handled with great care (Otieno et al., 2010). Acute ingestion of carbofuran, like other carbamates, through accidental exposure may lead to severe toxicity and fatalities to humans as well (Nicolopoulou-Stamati et al., 2016).

Carbofuran has the high toxicity to human and it is suspected an endocrine disruptor when ingested or exposed through orally and inhalation routes thus may pose a significant harm to those who are in immediate contact such as in manufacturing and formulating plants and in crop fields (Goad et al., 2004). Insecticidal and nematocidal carbamates, such as carbofuran have a high toxicity which is based on inhibition of acetylcholinesterase (ACHE), an enzyme responsible for hydrolysis of acetylcholine (ACH) to form choline and acetic acid (Das & Mukherjee, 1998; Bachman & Patterson, 1999). ACH is synthesized from choline and acetyl-CoA by enzyme choline acetyltransferase in the pre-synaptic neurons, and released into the synaptic cleft by exocytosis. Reversible binding of ACH to ACH receptor on the post-synaptic neuron will initiate a signal and neurotransmission continues. ACHE catches unbound ACH, hydrolyses it and the choline returns to the pre-synaptic neuron for reuse. The synthesis and hydrolysis process of ACH is illustrated in Figure 2.3. Carbofuran exposure can inhibit acute signs and symptoms to cholinesterase inhibitors due to blockade of acetylcholinesterase and subsequent build-up of acetylcholine and thus lead to overstimulation of cholinergic receptors in the central and peripheral nervous system (Bushnell & Moser, 2006). In mammals, cholinesterase inhibition caused by carbamates may be last for a short duration due to rapid metabolism and reversible inhibition (Morais et al., 2012). Albeit the indeterminate rapid process, early symptoms of carbofuran exposure may appear such as muscle weakness,

dizziness, sweating and slight body discomfort with headache, nausea, vomiting, abdominal pain and diarrhoea (Matsumura, 2012b).

Carbofuran is highly soluble in water which increases the environmental distress towards the aquatic lives (Campbell et al., 2004). According to De Melo Plese et al. (2005), aquatic ecosystems are particularly vulnerable to carbofuran pollution, as demonstrated by its groundwater ubiquity score (GUS) index of 4.5, which describes a relatively high risk of migration from soil of application areas to adjacent water bodies (De Melo Plese et al., 2005). Apart from that, a study carried out by Kawamoto and Makihata (2003), carbofuran derivatives namely carbosulfan, furathiocarb and benfuracarb are also manufactured and used as pesticides. These pesticides are found to degrade in soils and contaminate the tap water in Japan. More so, its metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran are also similarly poisonous to target and non-target species during degradation process *via* hydrolysis, redox, and microbial activity (Pessoa et al., 2011). Besides, a study done by Pant et al. (1995) claimed that carbofuran exposure could disrupt testicular morphology and alter the activities of enzyme associated with the testes cells. These impacts could be expected in developing reproductive organs if exposure occurs *in utero* or through mother's milk (Pant et al., 1995).



Figure 2.3: Synthesis and hydrolysis process of acetylcholine (CoA: Coenzyme A) (Pessoa et al., 2011)

2.4 Brinjal

Brinjal (*Solanum melongena*) is one of the most valuable vegetable and is commonly grown in many countries throughout Central, South, and South East Asia, some parts of Africa and Central America where hot and climates prevail (Kalawate & Dethe, 2012). Throughout the warm regions of the Far East, the brinjal is of great significance, rising extensively throughout India, Bangladesh, Pakistan, China, Philippines and Malaysia. In India, with the exception of higher altitudes, it is one of the most abundant, prominent and principal vegetable crops grown across the country (Kumar et al., 2008). Meanwhile in Malaysia, brinjal or known as "terung" is widely grown with a cumulative planting area of 2,579 ha, giving the production output of 40,418 metric tonnes in the year 2017 (DOA, 2017). This infers that brinjal is indeed a versatile crop that can be grown and available in the market throughout the year due to its adaptation to different agro-climatic regions.

This purple, green or white pendulous fruit is a member of the *Solanaceae* family and genetically similar to tomatoes and potatoes. Brinjal or eggplant is a well-known sub-tropical and tropical solanaceous crop. The name "brinjal" is common in Indian subcontinents and derived from Arabic and Sanskrit meanwhile "eggplant" was derived from the appearance of the fruit, which are white and resemble chicken eggs shape. Apart from that, it is also known as "aubergine" which is a French word in Europe, "melongene" in West Indies, "Guinea squash" in America and "patlican" in Turkey (Daunay, 2008; Naeem & Ugur, 2019). It is a seasonal but grown commercially as an annual crop. A variety of cultivars are grown depending on the colour, size and shape of fruit. Brinjal varies from its fruit shapes and colours, ranging from oval or egg-shaped; and from white, yellow, green to almost black through degrees of purple pigmentation. Some cultivars even display a gradient of colour from stem to base of the fruit (Sekara et al., 2007).

2.4.1 Usage and Potential of Brinjal

Brinjals are known for its rich source of abundant nutrients and their contents which all desirable for body growth. Due to its low calories and high phenolic contents, it is ranked as the most top ten vegetables that provide the healthiest food. Brinjal is a host of supplying various vitamins (vitamin A, B and C), iron, calcium, potassium, magnesium, and phytochemicals that contain phenolic components (caffeine and chlorogenic acid), and flavonoids (Kalawate & Dethe, 2012). Apart from that, brinjals are cooked in various ways such as baking, barbecuing, frying or pickling. They can also be pureed, flavoured and used as a dip or chutney as Mediterranean and Indian cuisines. In Indian cuisine, brinjals are used in curries and even made into soufflés (Ware, 2016).

Apart from the nutritional and agricultural value, it also comes with numerous amounts of therapeutic potential. Conventionally, brinjals are used in traditional Chinese medicine to stop intestinal bleeding. Besides that, parts of the plant also used as laxative (Halberstein, 2005). The fruit is also used as an antidote for mushroom poisoning. Meanwhile, in traditional Malay medicine, the ashes of the fruit are used in dry, hot poultices to treat haemorrhoids and ulcers where the root is pounded and applied inside the nostrils (Meyer et al., 2014).

According to Im et al. (2016), brinjal extracts have excellent healing effects on various disorders such as burns, asthma, high blood pressure, warts, inflammatory diseases, gastritis, stomatis, arthritis and stroke. In addition, mature brinjal fruits are used against stomachache, compress for swellings and splintered nipples (Hardiman, 2009; Salamon, 2014). Daunay (2008) also stated that dry brinjals are found to be effective in the treatment of stomach bloating, gas and piles treatment, while fresh fruits consumptions strengthen bones, control diabetes, prevent paralysis and an aid in gum problems. Brinjal provides a wide variety of specific secondary metabolites along with other compounds such as glycol-alkoloids, antioxidant compounds and vitamins that have played a vital role in maintaining good health. For instance, chlorogenic acid, a major phenolic compound found in fruit skin that acts as an anti-obesity, anti-inflammatory, anti-diabetic and as well as cardio-protective agents (Salamon, 2014; Naeem & Ugur, 2019). Chlorogenic acid also plays a role as anticarcinogenic agent by killing certain human cancer cells such as leukemia and lung cancer cells (Linnewiel-Hermoni et al., 2015). Consequently, brinjal is indeed an agronomically and economically essential vegetable with a significant foundation supply of nutrients which are vital for various pharmaceuticals purposes.

2.4.2 Brinjal Pest and Disease Management

Brinjal is a summer crop and it is cultivated in irrigated condition throughout the year. Therefore, they are vulnerable to damage due to a variety of pest organisms and insects from the nursery stage until harvesting (Kalawati et al., 2012). Among the insect pests infesting brinjal, the main ones are the shoot and fruit borer, (*Leucinodes orbonalis*), whitefly, (*Bemica tabaci*), leaf hopper, (*Amrasca biguttula biguttula*) and red spider mite (*Tetranychus macfurlanei*). The shoot and fruit borer are deemed as the greatest constraint since it affects the crop all year round. This pest has been recorded from all brinjal growing regions like India, Southeast Asia, USA and Germany. It is reported to attack brinjal shoot and fruit on all its stages of growth and this leads to yield loss of 70-92% due to pest infestation (Reddy & Sriniviasa, 2004) and enough to damage 4 to6 fruits for a single pest (Jagginavar et al., 2009). Due to loss of quality, the infested fruits are unsafe for consumption and thus will lose their market value.

Among the biotic factors, brinjal shoot and fruit borer is considered by far the most harmful pest of brinjal. The pests are active in temperate climate throughout the year and are able to lay eggs approximately 250 eggs one by one on developing fruits and young shoots of brinjal which cause a severe damage (Javed et al., 2017). Newly hatched larvae bore the petioles, midribs of large leaves and small tender shoots. The translocation of nutrients to shoots is impacted due to larval behaviour which will be unfit for consumption. Due to that, this will lead to shrinkage and drooping of the shoots, resulting in significantly reduced brinjal growth and size and number of fruits (Javed et al., 2007). Not only that, larvae can enter young fruits, create tunnels and begin feeding on internal tissues. The tunnels are obstructed with the excrement of the larvae and rendering the fruits unmarketable (Srinivasan, 2008). As mentioned, brinjal is a very pregnable vegetable which can be targeted by different insects. Various kinds of insecticides are recommended to control pests in order to obtain better growth of brinjal. The insecticides are normally applied before brinjal starts flowering (bud) until its harvesting period. Raza et al. (2018) reported that a lot of pesticides are used during brinjal cultivation in Bangladesh (Raza et al., 2018). To overcome this problem, the insecticides that are frequently used by the brinjal farmers are carbosulfan, emamectin benzoate, fipronil, abamectin, cypermethrin, thiamethoxam, chloraniliprole, acetamiprod, flubendiamide, envalerate and baizinon. These insecticides are approximately applied daily during brinjal harvesting season and some used day after day.

Eggplant farmers mostly rely on pesticides to combat this obnoxious pest. It is found that carbosulfan and flubendiamide are more efficient to poison these pests as compared to cypermethrin and abamectin (Latif et al., 2010). It is shown that carbamate pesticides such as carbosulfan reduced more than 80% shoot and fruit pest infestation as compared to control (Latif et al., 2010). However, the use of pesticides can be costly, and detrimental to human health and the environment. The indiscriminate use of pesticides allows the borer to be tolerant of such chemicals. As a result, it makes it impossible to manage this insect pest again economically with the same chemicals. Therefore, this study was undertaken with the objective to investigate the behaviour and disappearance rate of insecticides particularly carbofuran towards brinjal cultivation under temperate conditions.

2.5 Dissipation of Pesticides in Plant and Soil

It is important to understand the fate and behaviour of pesticide in plant and soil because plant acts as the transitional media whilst soil acts as an entering point to water resources and also the food chain (Fong et al., 1999). Pesticide introduced to the environment will undergo changes that led to structural alteration, detoxification, deactivation and finally disappearance of the active ingredients (Harrison & Sletten, 1990; Sposito, 2008).

Dissipation rate of pesticides is defined as the rate at which the pesticide active substance disappears from the measured part of the plant due to various processes including volatilization, hydrolysis, photodegradation, chemical and microbial. The rate of dissipation are often measured using field studies, and they are usually expressed as the pesticide half-life ($t_{1/2}$); the half-life is defined as the time needed for pesticide residue level to reduce to half of the initial concentration after application. The dissipation of pesticides is often governed by the pesticides formulation, physio-chemical properties of pesticides (solubility, ionisation, adsorption and affinity) (Lewis & Tzilivakis, 2017), climate conditions (temperature, sunshine, rainfall, humidity) and plant physiology (Fan et al., 2013; Fantke & Juraske, 2013).

Pesticides play an important role in improving food quality as well as controlling various kinds of pests that could harm crops and livestock yields (Cooper & Dobson, 2007; Kazemi et al., 2012). The dissipation rate of a pesticide is the central parameter in representing its environmental fate. The residual dissipation rate could be affected by species cultivated (crop formation, cuticle characteristics, growth rate, pH dependency, etc), application parameters, climatic conditions and mode of action (Minelli et al., 1996; Lin et al., 2001; Rahman et al., 2015). The degradation of pesticide also depends on the abiotic environmental conditions, variation in plant species and/or microbial community, characteristic of pesticide and all biochemical processes involved (Van Eerd et al., 2003).

2.5.1 Dissipation Mechanisms of Pesticides in Plants

Pesticides may be dissipated *via* various pathways including chemical degradation, volatilisation, biotransformation, plant growth dilution and wash-off by rainfall degradation as illustrated in Figure 2.4. (Fong et al., 1999; Laabs et al., 2002; Katagi, 2004). These processes may occur individually or simultaneously, governed by environmental conditions (humidity, rainfall, temperature, UV radiation), plant morphology (waxy peel, hairy, rough or smooth surface, water and lipid content), pesticides formulation and dosage applied (Juraske et al., 2007; Fantke & Juraske, 2013).



Figure 2.4: Dissipation Pathway of Pesticide in Plant (Fanke & Juraske, 2013)
Pesticide deposits on plant surface may undergo chemical degradation through oxidation, reduction, hydrolysis and conjugation processes (Fernandez-Alvarez et al., 2007; Fantke & Juraske, 2013). These processes can be categorised into three phases with oxidative, reductive and hydrolysation occur in the initial phase, followed by conjugative and formation of secondary conjugates and finally formation of metabolites (Fantke & Juraske, 2013).

Photodegradation of pesticides also involves chemical reactions, such as oxidation, reductions, catalytic hydrolysation and bond-cleavage which occur in the presence of sunlight radiation (Fernandez-Alvarez et al., 2007; Fantke & Juraske, 2013). The free radicals and photon energy from sunlight will initiate organic reactions such as decarboxylation and oxidation of reactive oxygen species (Burrows et al., 2002; Katagi, 2004).

Pesticides volatilisation takes place on outer layer of plant. Volatilisation of pesticides is influenced by their volatility (vapour pressure), temperature and wind velocity (Spencer et al., 1973; Rüdel, 1997). Volatilisation usually occurs during foliage application. It will continue for several hours after application depending on the surrounding temperature, wind and pesticide formulation (Juraske et al., 2008).

Pesticides that break down and up-taken by plant may undergo biotransformation, which occurs *via* plant metabolism and other biotic processes, intervened by plants and microorganisms (Hoyle & Arthur, 2000). As soon as pesticide enters the inner part of plant, they will undergo biological transformation which involves enzyme as catalyst (Juraske et al., 2008). This enzymatic reaction will modify and transform the structure and toxicological properties of pesticides (Hoyle & Arthur, 2000). Biotransformation is considered to be the route of pesticides detoxification and metabolism in vegetation (Van et al., 2003).

2.5.2 Dissipation Mechanisms of Pesticides in Soil

Modern agriculture is highly dependent on the use of pesticides to meet the ever growing demand for food and fibre. While pesticides are invaluable for raising the quantity and quality of food resources and for securing society through improved health and higher living standards, their movement off-site and adverse effects on surface and groundwater quality are of environmental concern. Sorption and degradation are two governing processes which determine the distribution and persistence of pesticides once introduced into the soil. Pesticides may be degraded by biological and/or abiotic means including photolysis or volatilisation. The dissipation pathway of pesticide in soil can be summarized in Figure 2.5 (Sarmah et al., 2009).



Figure 2.5: Dissipation pathway of pesticide in soil (Sarmah et al., 2009)

Volatilisation is a process that leads to transfer of pesticides from soil or plant compartments into the atmosphere (Spencer, 1991). Pesticides are low-volatile to semi-volatile compounds, thus they can be distributed in all phases: solid, liquids and gases (Taylor & Spencer, 1990; Spencer et al., 1995). Volatilisation may cause pesticides dispersion and translocation to distant locations. Pesticides volatility depends profoundly on their vapour pressure; pesticides with high vapour pressure will be volatilised easily. In addition, pesticides loss due to volatilisation will increase with increasing temperature. This is supported by the findings of Alvarez-Benedi et al. (1999) where the cumulative loss was about 6% at 25 °C versus less than 1% at 15 °C in sandy soil).

Adsorption of pesticide to soil particles will lead to the retention of pesticides in soil. The retention may control their bioavailability, leaching potential, degradation and volatilisation (Laabs et al., 2002; Sposito, 2008). A few mechanisms are involved in the adsorption of pesticides such as *Van der Waals* interaction, cationic and anionic bonding, hydrogen bonding, charge transfer, ligand exchange, direct and induced ion dipole, dipole-dipole interaction and chemisorption (Gevao et al., 2000; Sposito, 2008). These mechanisms are controlled by the natural properties and components of soil (soil pH, soil moisture, soil organic matter and clay and mineral contents) (Sposito, 2008).

One of the most critical pathways of dissipation is degradation. Degradation process may alter and modify the parent compound forming either non-toxic metabolites (CO_2 or H_2O) or more toxic and persistent compounds (Kah et al., 2007). Degradation of pesticides in soil may occur *via* photodegradation, chemical degradation (hydrolysis, ionisation, oxidation and reduction) and biological degradation (Topp et al., 1997; Burrows et al., 2002). Through these degradation processes, a number of metabolites will be produced from a single pesticide.

Photodegradation or photolysis is one of the most destructive pathways that takes place in the presence of reactive species, initiated by sunlight (Frank et al., 2002; Fernandez-Alvarez et al., 2007). This process involves breaking down of chemical bonds as sunlight imparts photolytic energy upon the chemicals (Burrows, 2002; Katagi, 2004). Frank et al. (2002) stated that the formation of reactive ions (singlet oxygen and hydroxyl radical) was due to the exposure of the soil to sunlight. Photolysis was found to be effective only in the upper layer (< 2.0 mm depth) where the amount of extractable materials decreased with increasing soil depth (Frank et al., 2002).

On the other hand, chemical degradation such as hydrolysis, oxidation-reduction (redox) and ionisation usually takes place in the deeper layer of soil where microbial population is limited (Racke, 2003). These reactions are pH dependent where the chemical degradation is favoured when pH increases (Gevao et al., 2000; Andreu & Picó, 2004). However, chemical degradation may cause soil pollution and acidification and its consequences include mobilization of harmful compounds, increase of soil salinization, nutrient depletion and decrease of natural buffering capacity (Lal, 2001; Racke, 2003).

Biodegradation of pesticides occurs through the activities of microorganisms and biomass population (Racke & Coats, 1990). It involves the breakdown of pesticides by microorganisms such as bacteria and fungi where pesticides are used as an energy source for growth (Matsumura, 2012a). The breakdown products are usually non-toxic such as carbon dioxide, water and mineral (Jebellie et al., 1996; Kah et al., 2007). The rate of biodegradation is influenced by the soil factors such as moisture, aeration, nutrient availability, presence of inhibitor, pH and temperature. Essentially, the degradation is enhanced in soil with pH ranges from 5.5 to 8.0, attaining optimal degradation at pH 7. The degradation also tends to increase with temperature (Shelton & Parkin, 1991; Sparks, 2003).

The dissipation of pesticides could also occur *via* washing off by rainfall. The loss of pesticides may vary appreciably depending on rainfall intensity, time of rainfall after initial application, the crop treated, pesticide solubility and formulation used (Cohen & Steinmetz, 1986; McDowell et al., 1987; Isensee & Sadeghi, 1994). However, the potential of pesticide being absorbed in soil or plant may reduce especially after precipitation (McDowell et al.,

1985, 1987). A study on fenvalerate by McDowell et al. (1987) on cotton plants reported that 38% of the pesticide was lost, two hours after receiving 25 mm rain. Meanwhile, a study carried out by Cohen and Steinmetz (1986) recorded 35 – 61% loss of flucythrinate, fenvalerate and azinphosmethyl and over 90% loss of methyl parathion after 25 mm of stimulated rainfall was applied. According to Sigua et al. (1993), pesticide prone to be washed from dead plant tissue (crop residue from previous years) compared to freshly harvested vegetation leading to pesticide runoff and leaching during rainfall (Reddy et al., 1994). Therefore, in this context, the persistence, efficacy and runoff losses of pesticide are governed by the amount of elapsed time between application of pesticide and initial rainfall.

2.5.3 Factors Controlling Dissipation of Pesticides

Pesticides are considered indispensable for the production of grains, fruits and vegetables. Pesticides enter the soil *via* several routes such as direct application, washed-off from treated foliar spray or spray drift during application. Pesticides which are applied directly to the soil may be washed off into the water body or may percolate to lower soil layers and groundwater (Ciglasch et al., 2006). Once released to the soil, sorption and degradation are two major governing processes that determine the distribution and persistence of pesticides.

Pesticides can undergo degradation either through biological and/or abiotic pathways. Microbial transformation can take place directly through metabolic processes such as mineralization, co-metabolism, conjugation and accumulation (Bollag & Liu, 1990; Andreü & Pico, 2004). Apart from the chemical properties of pesticides, soil and environmental factors such as pH, clay and organic matter content, moisture content, and temperature also affect the rate of degradation (Roy et al., 2000; Sarmah et al., 2004). In addition, the fate and behaviour of pesticides also depend on the climate condition, geographical and physicochemical properties of pesticides (Racke et al., 1996; Bloomfield et al., 2006).

As mentioned, climate condition is one of the main factors affecting the dissipation of pesticides. It has a potential effect on the environmental fate and behaviour of pesticide by permuting the fundamental mechanisms of partitioning between the environmental compartments as well as giving impact on the pesticides use (Noyes et al., 2009). Consequently, farmers may have to apply more pesticide in case of lower residue due to climate change (Bloomfield et al., 2006). The degradation of pesticides in soil is also controlled by pesticide transport such as volatilization, wash off and leaching processes. Pesticides uptake into plants is governed by soil properties and plant growth, both influenced by climate change. Typically, most food crops are sensitive under severe conditions such as high temperature or extreme precipitation thus it will induce lower transpiration and affect the plant productivity (Rosenzweig et al., 2001; Kookana et al., 2010). Generally, the dissipation of pesticides is mainly controlled by rainfall seasonality, intensity and temperature changes whereby an increase of temperature may require higher application of pesticides onto plants.

Besides that, soil organic matter is also liable for the dissipation of pesticides. Organic matter is the foundation for healthy and productive soils. Its presence has been positively correlated with the retention and persistency of pesticides in soil (Sposito, 2008). Humic substance is a major component of soil organic matter. It is fractionated into humic acids and fulvic acids that attribute to polydispersity and polyelectrolytic properties. The presence of various chemically-reactive functional groups, free radical moieties and hydrophilic and hydrophobic sites are involved in the interactions with organic pesticides (Senesi, 1992). Such interactions include adsorption, partitioning and solubilisation, hydrolysis, dealkylation and photosensitization (Senesi & Miano, 1995).

A study by Gunasekara and Xing (2003) stated that the sorption and desorption of organic compounds in soil are primarily regulated by soil organic matter. Mineral and/or organic component in soil is in charge of the sorption interactions of pesticides. According to Spark & Swift (2002), if the soils have higher organic matter levels (> 5%), the sorption interactions with pesticides is insignificant. Meanwhile, in soils with low organic matter, the mobility of pesticides is governed by the active components of inorganic fraction such as claysized fraction (Barriuso et al., 1992; Baskaran et al., 1996; Spark & Swift, 2002). Therefore, the content of organic matter in soil could exert influence on the residual concentration of pesticides enhancing soil adsorption competency and at the same time reducing pesticide bioavailability.

Soil water content or soil moisture is another important factor which governs pesticide dissipation in soil (Frank et al., 2002). According to Jebellie et al. (1996), the soil moisture is important to enhance biomass activity. A study done by Swarcewicz and Gregorczyk (2012) stated that the soil moisture affects the dissipation and absorption of pesticide depending on the type of soil and pesticide used. For polar pesticides, high soil water content may enhance dissipation through leaching (Laabs et al., 2012). For non-polar pesticides, the adsorption decreases when the soil water content increases because at a higher moisture levels, water molecules tend to compete with pesticides for adsorption sites on the soil colloids (Smith et al., 1995; Laabs et al., 2002; Ismail et al., 2012). For most pesticides, water acts as a hydrolytic agent that controls hydrolytic and photolytic degradation (Jebellie et al., 1996; Frank et al., 2002).

Soil pH also affects the pesticide adsorption either through abiotic and biotic degradation processes (Harris et al., 1994). The sorptive behaviour of pesticide on clay and organic surfaces is influenced by soil pH that eventually affect the chemical speciation, mobility

and bioavailability of pesticides (Hussain et al., 1994). Furthermore, the effect of soil pH on degradation of a pesticide depends greatly on whether a compound is susceptible to alkaline or acid hydrolysis. Evidently, the degradation of most pesticides has shown to be faster at high pH (Given & Dierberg, 1985; Racke et al., 1996; Singh et al., 2003a; Singh et al., 2003b). The microbial degradation is normally restricted at high pH (pH > 7). Singh et al. (2003b) reported that the degradation of fenamiphos was slowed down in acidic soils (pH 4.7 to 6.7) while in pH 6.7 to 6.8, rapid degradation was observed in which plant nematods was no longer biologically active.

2.6 Dissipation Mechanisms of Carbofuran

The degradation pathways and factors affecting the behaviour of carbofuran were studied (De Bertrand & Barcelo, 1991; Mora et al., 1996; Trabue et al., 2001). Carbofuran degradation and metabolism could occur through volatilisation, leaching, surface runoff, as well as plant uptake (Mora et al., 1996). These processes often lead to breakdown of carbofuran at the benylix carbon forming secondary metabolites such as 3-ketocarbofuran, 3-hydroxycarbofuran phenol and 3-ketocarbofuran phenol (Figure 2.6).

Base-catalysed hydrolysis is the major degradation pathway of carbofuran in water and soil where it yields carbofuran phenol as its secondary metabolite (Yu et al., 1974; Seiber et al., 1978; Tablebi & Walker, 1993). Other possible pathways include oxidation, methylation, chlorination and rearrangement (Raha & Das, 1990). Carbofuran is highly soluble in water; therefore it can be easily leached due to its high mobility factor (Lalah et al., 2001). Water serves as a reactant or provides a medium for chemical degradation reactions of carbofuran in soil. Lalah et al. (2001) found that majority of carbofuran residues persisted on the submerged

soil surface during 111 days of dissipation analysis due to its high water solubility. This suggested the feasibility of contamination with carbofuran, particularly in nearby body of water which flowed from the irrigation canals through the farm. However, the study also observed that carbofuran was found to leach extensively at the same field site even under dry soil conditions. Therefore, carbofuran undergoes hydrolysis at the benzylic carbon to produce its metabolites in both flooded and non-flooded soils and the rate of hydrolysis is governed with the soil pH (Campbell et al., 2004).

Photodegradation of carbofuran was widely studied suggesting that exposure to sunlight or other light sources could affect the dissipation of carbofuran. De Betrand and Barcelo (1991) observed that carbofuran was rapidly degraded under unfiltered mercury lamp compared to xenon arc lamp (90 minutes and 120 minutes respectively). A study conducted by Campbell et al. (2004) concluded that photodegradation of carbofuran was 31 times faster in seawater than in distilled water with half-life of 0.1 hour and 3.1 hours, respectively. Farahani et al. (2012) stated that the half-life of carbofuran in river water is longer than the half-life in sea water when they are exposed to sunlight.

Apart from that, the adsorption effect in soil is also one of the degradation mechanisms of carbofuran. It is responsible for the dissipation of carbofuran under darkness (Farahani et al., 2007). Adsorption is the cumulative accumulation of substance at a substance between a solid phase and an aqueous solution phase (Bermúdez-Causo et al., 2012). The amount of chemical being absorbed on the solid surface of soil may influence the physical and chemical propertied of soil. The organic carbon content, clay, cation exchange ability (CEC) and pH seem to regulate the adsorption of carbofuran in both tropical and temperate soils (Bermúdez-Couso et al., 2011). Adsorption in soil prevents pesticides from occurring in surface and ground waters, however impedes the volatilization, and biodegradation, meanwhile desorption in the soil

promotes drainage and leaching into water bodies which makes the pesticide accessible to soil microorganisms thus facilitates biodegraration. A study by Bermudez-Causo et al. (2010) found that the carbofuran sorption in soil was more than 80% due to fast adsorption processes governed by intraparticle contents which in favourable with the high organic carbon content in soil. Khan et al. (2010) asserted that organic matter content above 2% promotes the adsorption of pesticides onto organic components meanwhile the mobility of pesticides in soil with low organic matter contents to be largely governed by inorganic fractions or clay (Yazgan et al., 2005).



a) 3-hydroxycarbofuran



c) carbofuran phenol



b) 3-ketocarbofuran



d) 3-hydroxycarbofuran phenol



e) 3-ketocarbofuran phenol

Figure 2.6: Molecular structures of metabolites of carbofuran (Mora et al., 1995)

Biodegradation is the main pathway of dissipation for most insecticides in soils including carbofuran. Carbofuran may be targeted by microorganisms in several possible sites and cleave of ester or amide linkages in carbofuran to give carbofuran phenol, 3-keto-7carbofuran phenol or 3-hydoxy-7-carbofyran phenol. Numerous studies have evidenced the ability of soil microorganisms in using carbofuran and its degradation products as source of carbon and energy (Shelton and Parkin, 1991; Slaoui et al., 2007; Chanika et al., 2011). A research performed by Karpouzas et al. (2001) stated that the degradation rate of carbofuran is higher in sub-soil compared to top-soil due to the interactive effects between microbial activities and organic matter contents in different soil layers. The study also reported that an amount as low as 0.1 mg kg⁻¹ was adequate to activate the surface soil biota to accelerate the degradation of carbofuran even one year after the initial treatment. Therefore, the application of carbofuran in soil will stimulate the growth and activities of bacteria, actinomycetes, fungi, N₂-fixing bacteria and phosphate microorganisms which will result in rapid degradation of carbofuran. Moreover, Lalah et al. (2001) stated the microorganism Actinomyces is responsible for converting carbofuran to CO_2 . In flooded soil where oxygen (O_2) is minimize, it was suggested that the hydrolysis of carbofuran is mainly chemical but subsequent breakdown of carbofuran phenol takes place by mircoorganisms.

There are several factors that play vital roles in dissipation of carbofuran for example soil pH, moisture and temperature and organic matter content. Singh et al. (1990) reported that the degradation of carbofuran is accelerated at 35 °C compared to 25 °C. Mora et al. (1996) also stated that the concentration of carbofuran decreases after 14 days at 30 °C and after 4 to 6 days at 40 °C. This could be explained by the proliferation of microorganisms with increased temperature (Yen et al., 1997; Mohamed et al., 2013).

The soil moisture also gives a substantial influence in the degradation of carbofuran where the higher the soil moisture, the shorter is the half-life. This is because under high moisture content (100% flooded condition), it gives rise to anaerobic conditions encouraging hydrolysis of carbofuran yielding carbofuran phenol as the main metabolite (Getzin, 1973; Rajagopal et al., 1984). This is supported by the study of Kale et al. (2001) where more bounded residues were formed under moist than flooded soils.

The half-life of carbofuran is highly dependent on pH, where the rate of degradation increases as the pH increases (Seiber et al., 1978). Soil pH influences the ionizability of fulvic acid functional groups such as carboxylic acid (-COOH) and hydroxyl (-OH) thus it is an important control factor in the hydrolysis process in pesticides (Lalah et al., 2001). It was reported that the hydrolysis of carbofuran was faster at pH 10 than at pH 7 where the half-lives were 1.2 hours and 864 hours, respectively (Seiber et al., 1978). Getzin (1973) also stated that the half-life of carbofuran in soil ranging from 3 weeks up to more than 50 weeks depending on the soil pH. The breakdown of carbofuran is much faster (10-fold quicker) in alkaline soils than acidic soils. Therefore, hydrolysis is the major pathway in alkaline soil while microbial and chemical mechanisms predominate in acidic soil (Getzin, 1973).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Chemical and Reagent

Carbofuran standard was purchased from Dr. Ehrenstorfer GmbH. Carbofuran granules (AGRITOX 3G) were purchased from Hextar Chemicals Sdn. Bhd. Analytical and residue grades of acetonitrile, sodium chloride, acetone, glacial acetic acid and anhydrous magnesium sulphate were purchased from J.T Baker, Phillipsburg, USA. Florisil (2% deactivated) was used as a sorbent for extraction. A stock standard solution of carbofuran and 3-ketocarbofuran at 500 mg/L was prepared in acetone. The stock solution was diluted to standard solutions of 0.001, 0.1, 0.5, 1.0, 10 and 50 mg/L and stored at 4 °C in the dark.

3.2 Apparatus and Instrumentations

A Robot Coupe CL50E (USA) food chopper was used to homogenise the vegetable samples while centrifugation of the sample extracts was performed using Thermo Jouan Model B4i (Thermo Electron Corporation, USA) multifunctional centrifuge. An Agilent Model 7000 (Agilent Technology Incoporation, USA) gas chromatography equipped with mass spectrometry (GC-MS/MS) was used to determine the pesticides present in the samples.

3.3 Soil Analysis

The soil was sent for analysis which was conducted in Chemistry Laboratory at Agricultural Research Centre, Semongok, Sarawak. Prior to planting, Semongok soil samples were collected and analysed for its pH, conductivity, exchangeable base cations, cation exchangeable capacity (CEC), particle size distribution, soil total organic carbon, soil nitrogen and soil moisture content. The soil analysis was done at the depth 0 - 15 cm and 15 - 30 cm. Every analysis was carried out along with blank soil samples as a reference standard.

3.3.1 pH and Conductivity

The pH and conductivity were measured using Thermo Scientific Orion Star A2150, Orion Star A215 pH/Conductivity Benchtop Meter (USA) on a suspension of soil in water. A 2:5 soil:solution ratio and 1:5 soil:solution suspension was used to measure the soil pH and conductivity respectively. 10 g of soil was weighed into a bottle and 25 mL of water was add, stopper and left overnight (more than 16 hours). The electrode was washed thoroughly with distilled water between samples. The settings of pH and conductivity meter were checked before, during and at the end of a run samples with buffer solutions before, during and again after sample run. The pH and conductivity of soil were read and recorded accordingly.

3.3.2 Particle Size Distribution

Meanwhile, the particle size distributions in soils were analysed using hydrometer method. The particle size analysis was done by dispersion of soil aggregate into discrete units after the destruction of organic matter and the separation of particles according to size limits by sieving and sedimentation. The sedimentation or hydrometer method is used to determine the particle-size distribution (gradation) of the material that is finer than the 75-µm sieve and larger than about 0.2- μ m. The analysis is performed on material passing the 2.0-mm or finer sieve and the results are presented as the mass percent finer versus the log of the particle diameter. Proportions are represented by stated class sizes: sand ranging from 2000 – 50 μ m; silt ranging from 50 – 2.0 μ m and clay < 2.0 μ m and those stated by the USDA Soil Survey and Canadian Soil Survey Committee (Huluka & Miller, 2014). The soil sampling were then categorised into clay, silt, fine and coarse sand.

3.3.3 Exchangeable Base Cations

Exchangeable base cations of calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) were determined by leaching the soil samples first with ammonium acetate solution buffered to pH 7 (Dewis & Freitas, 1970) and determined by using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) – Avio 200 (USA). Subsequently, according to Fallavier et al. (1985), the cation exchange capacity (CEC) was analysed by using ammonium acetate method and a discrete auto-analyzer. This analysis was conducted directly after the exchangeable cations (Ca, Mg, K, and Na) extraction. The remaining soil from exchangeable cations extraction were then analysed for CEC determination by using an auto-analyzer (SmartChem 200, Unity Scientific, USA).

3.3.4 Soil Total Organic Carbon

The soil total organic carbon was determined using the combustion method which was acquired from Schwartz (1995). The instrument (LECO RC-412 Multiphase Carbon Analyzer, American Laboratory Trading, USA) consists of a heat resistant quartz tube furnace and three high selective infra-red cells for CO_2 / H_2O monitoring. It allows the temperature at which they oxidize or volatize. Therefore, in an oxygen environment, carbon is oxidized to carbon dioxide

by combustion and detected by an infra-red cell. The temperature of the furnace was first set to 1000 °C and was left for an hour. Several soil blanks were analyzed until the reading stabilized. After that, the furnace was set to 550 °C and the soil sample was combusted for 5 hours to determine the total organic carbon in soil.

3.3.5 Soil Total Nitrogen

The soil total nitrogen was analysed by Dumas combustion method. In this method, the soil sample was combusted at 950 °C in pure oxygen environment by using LECO FP-528 Nitrogen Analyzer (American Laboratory Trading, USA). This leads to the release of substances such as carbon dioxide, water, nitrogen dioxide and, above all, nitrogen as several oxides (NyOx). The combustion products are collected and allowed to equilibrate. The gas mixture is passed over hot copper to remove any oxygen and convert nitrogen oxides into molecular nitrogen. The sample is passed through traps that remove water and carbon dioxide. Nitrous oxides were reduced to pure nitrogen (N₂) which was measured by a thermal conductivity detector. The results were displayed as percentage (%) of nitrogen in the computer. Soil moisture content determination was conducted by using air-dry soil samples. To express the results in oven-dry basis, the analysis results is multiple by moisture factor. This moisture factor is the ratio of the weight of air-dry soil and its weight after drying in the oven for 105 °C for 8 hours and left overnight to cool down. The weights of crucibles containing the soil samples were recorded.

3.4 Field Experiment

Brinjal plants (Solanum melongena) were planted in the field at Semongok Agricultural Research Centre (ARC), Sarawak (N 01°23'05.9", E 110°19'44.7). Brinjals were grown from its seeds and transplanted to the open field. The seeds were sown directly into polybags (10 cm × 12 cm) filled with topsoil. A total of 6 plots with similar size were set up; the vegetation were removed and the plots were ploughed with addition of 1 kgm⁻² chicken manure, 0.1 kgm⁻² dolomite and 0.02 kgm⁻² of compound fertiliser. The seedlings were planted in 6 rows and 27 columns with a spacing of 50 cm between seedlings yielding a total of 162 plants. The seedling were similarly left for acclimatization and carbofuran granules were only applied (the recommended dosage: 30 kg/ha) when plants began to bear fruits. Three beds were applied with carbofuran whilst three others serve as the control. The plants were watered daily, except rainy days. The temperature, humidity and precipitation data was provided by the Meteorology Department at Semongok Agricultural Research Centre. Figure 3.1 shows the photographs of the field experiments for brinjal. The subjected to analysis including pH, conductivity, exchangeable base cations, cation exchangeable capacity, particle size distribution, soil total organic carbon, soiloil nitrogen and soil moisture content prior to amendment and treatment with carbofuran (Pansu & Gautheyrou, 2007).



(a) Week 1



(b) Week 5

Figure 3.1: Photographs of field experiment for brinjal

3.4.1 Weather Condition

Temperature and relative humidity throughout the experimental period was collected from Semongok Agricultural Research Centre by using an Integrated Sensor Suite (ISS). The rainfall data was collected by using a rain gauge which is a meteorological instrument for measuring the amount of rainfall amounts fallen.

3.4.2 Samples Collection

One brinjal fruit was harvested and weighed from each bed on Day 0, upon application of carbofuran. Subsequent to Day 0, brinjal plants were sampled at an interval of three days for the first four weeks and at an interval of seven days for the succeeding three weeks with 3 replicates from each planting bed with and without carbofuran, respectively. The destructive sampling technique was applied where the whole plants were harvested. The soil leaves and fruits were collected for analysis of carbofuran and its secondary metabolite (3-ketocarbofuran). For soil, approximately 0.5 kg of topsoil was collected from around the plants and the corresponding weight of the brinjal fruit was recorded. The fruit and leaves were diced, homogenized, and stored in a freezer at -20 °C until analysis. The soil samples were air-dried, ground and sieved through 2 mm sieve to remove debris and roots before subjected to chemical and physical analyses (Chai et al., 2008).

3.4.3 Sample Preparation

The pesticides were extracted from soil and brinjal samples (fruits and leaves) using the established method of QuEChERS (Payá et al., 2007). Ten grams of sample was weighed into a 50 mL Teflon centrifuge tube. Twenty millilitres of acidified acetonitrile (1% acetic acid) was added to the samples and shaken vigorously for 1 min by hand followed by vortex mixing for

1 min. Six gram of anhydrous magnesium sulphate and 1.5 g of sodium chloride were added and vortexed vigorously for 1 min. The extract was later centrifuged for 2500 rcf for 1 min. To determine the residue of carbofuran, the extract was cleaned up using 0.2 g deactivated Florisil packed in a glass pasteur pipette for clean-up. The eluent was left to dry, added with 2 mL acetone and transferred to a vial.

The sample was analyzed in triplicates for carbofuran and its metabolite using a Gas Chromatography Mass Spectrometer with tandem mass spectrometry (GC-MS/MS Agilent Model 7000). A column of HP-5MS 5% phenyl methyl Silox (30 m × 0.25 μ m × 0.25 μ m obtained from J and W Scientific, USA and was used with helium and nitrogen as carrier gas at a flow rates of 2.230 mL/min and 1.500 mL/min, respectively. The column temperature was maintained at 70 °C for 2 mins and subsequently increased to 150 °C at 25 °C/min. The temperature was then increased to 200 °C at 25 °C/min and further to 280 °C at 65 °C/min. The temperature was held constant for 2 mins. The concentration is reported in mg/kg fresh weight.

3.5 Method Validation

Soil samples were spiked with carbofuran and 3-ketocarbofuran standards to attain concentrations ranging between 0.01 and 1.0 mg/kg, respectively. The spiked samples were mixed and left for 15 mins before extraction and analysis with GC-MS/MS. The recovery performance was calculated as the percentage of experimental concentration over the expected concentration. The blank samples were also analyzed. The limit of detection (LOD) was evaluated based on the lowest recoverable concentration.

3.6 Degradation Study

3.6.1 Experimental Design

The degradation of carbofuran in sterilized soil under the effects of three factors, temperature, moisture and pH was studied using the response surface methodology (RSM). Table 3.1 summarizes the coded and uncoded level of moisture, pH and temperature studied. The variables were coded according to the Equation 3.1.

$$x = 2 \frac{(X - X_{mid \ value})}{\Delta X}$$
 Equation 3.1

where $X_{mid \ value}$ is middle value of X in the data set and ΔX is the range of parameter from its minimum to its maximum value of X, respectively.

The range of pH (pH 4 – 10), moisture (30 – 70%) and temperature (30 – 70 °C) were designed based on the tropical soil properties. The average pH, moisture and temperature of soil in tropical region are pH 7, 50% moisture and 30°C respectively, therefore maximum and minimum value of these three parameters are chosen for independent variables which are presented in Table 3.1 and 3.2 respectively (Muche et al., 2015). The soil was incubated at different temperatures with its pH adjusted with NaOH and HNO₃ whilst moisture was added gravimetrically to the air dried soil. The experiment was designed based on circumscribed central composite design as shown in Table 3.2.

Factors		Coded levels			
		-1	0	1	
рН	X1	4	7	10	
Moisture	X2	30	50	70	
Temperature	X3	30	50	70	

Table 3.1:Coded and uncoded level of moisture, pH ans temperature

Table 3.2:	The experimental design according to circumscribed central composite design
	(CCD) – Coded and uncoded variables

Experiment	Experiment design			Experiment design		
number	(Coded)			(Uncoded)		
(n)	X1	X2	X3	X 1	X2	X3
1	0	0	0	7	50	50
2	0	0	0	7	50	50
3	0	0	0	7	50	50
4	0	0	0	7	50	50
5	0	0	0	7	50	50
6	0	0	0	7	50	50
7	0	0	0	7	50	50
8	0	0	0	7	50	50
9	0	0	0	7	50	50

Experiment	Experiment design			Experiment design		
number	(Coded)			(Uncoded)		
(n)	X1	X2	X3	X1	X2	X3
10	0	0	0	7	50	50
11	0	-1.68	0	7	16	50
12	0	1.68	0	7	82	50
13	-1.68	0	0	2	50	50
14	1.68	0	0	12	50	50
15	0	0	-1.68	7	50	16
16	0	0	1.68	7	50	82
17	-1	-1	-1	4	30	30
18	-1	1	-1	4	70	30
19	-1	-1	1	4	30	70
20	-1	1	1	4	70	70
21	1	-1	-1	10	30	30
22	1	1	-1	10	70	30
23	1	-1	1	10	30	70
24	1	1	1	10	70	70

Table 3.2continued

Ten grams of dry soil was weighed into 50 mL glass vials and autoclaved for 1 hour for 121 °C. The sterilized soil was spiked with 100 μ L of 500 mg/L carbofuran to attain a concentration of 5 mg/kg. The samples were then incubated according to the experimental design in Table 3.2 and Table 3.3 for 24 hours. The concentrations of carbofuran after incubation were analysed in triplicates. The residues of carbofuran were extracted from the soil samples according to QuEChERS method mentioned in Section 3.3.4. The percentage of degradation was calculated as the percentage of carbofuran concentration after incubation against the concentration before incubation as follows (Equation 3.2):

Percentage of degradation

Equation 3.2

$$= \frac{\text{Concentration after incubation}}{\text{Concentration before incubation}} \times 100\%$$

3.6.2 Response Surface Methodology (RSM)

The design matrix and the corresponding percentage of carbofuran degraded were subjected to Matlab 2015 to derive the regression according to linear, interaction and quadratic models. The model suitability was assessed based on the root mean squared error (RMSE) and the regression coefficients - R^2 and adjusted R^2 (Equation 3.3 to Equation 3.5). The RSME indicates the prediction performance; the smaller the RMSE, the closer the model predicts the concentration of carbofuran. Correspondingly, R^2 and adjusted R^2 denote the model quality; values converging 1.0 postulate a good agreement between the predicted and experimental values. The optimum conditions for maximum degradation was then determined and verified experimentally. The regression equations of linear, interaction, and quadratic models are shown in Equation 3.6 to Equation 3.8 respectively.

$$R^{2} = 1 - \frac{\sum_{i=1}^{I} (y - \hat{y})^{2}}{\sum_{i=1}^{I} (y - \bar{y})^{2}}$$
 Equation 3.3

$$Adj R^2 = 1 - \frac{(1 - R^2)(n - 1)}{n - P - 1}$$
 Equation 3.4

$$RMSE = \sqrt{\frac{\sum_{i=1}^{I} (y - \hat{y})^2}{n - P}}$$
 Equation 3.5

where n = number of experiments; P = number of predictions

$$y = b_o + b_1 x_1 + b_2 x_2 + b_3 x_3 + k$$

(Linear model)
Equation 3.6

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + k$$
Equation 3.7

(Interaction model)

$$y = b_o + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2$$

Equation 3.8
$$+ b_{33} x_3^2 + k$$

where y = predicted response; x1, x2 and x3 = coded factors; b = coefficients; k = offset

term

3.7 **Estimation of Half-life** (t_{1/2})

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The half-life of carbofuran under the conditions optimum for dissipation of carbofuran was assessed over 24 hrs. The half-life was estimated on the first order kinetic reaction of Y = Ae^{-kt} , where Y is the residue concentration at time, t (day), A is the initial residue concentration and k is the dissipation rate constant (day⁻¹). From the experimental results, the natural log of the residue concentration, *Y* at sampling time, *t* was determined. The linear regression of ln (*Y*) versus *t* was modelled yielding $\ln(Y) = -\ln(A) - kt$. The half-life $(t_{1/2})$ was estimated from the equation of $t_{1/2} = \frac{0.693}{k}$. This regression was also applied to estimate the half-life of carbofuran under field condition (Fanke & Juraske, 2013).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Method Validation

Method validation was appraised prior to field trials and degradation study under laboratory settings. The method was validated to ensure its quality, consistency and sensitivity to reliably determine the residues present in brinjal and soil (Eurachem, 1998). Method validation was carried out to confirm the limit of detection (LOD), accuracy and precision of the simplified QuEChERS method for determination of carbofuran in brinjal and soil. Accuracy and precision were calculated based on the recovery percentage (n=3) (Lehotay et al., 2005; Kolberg et al., 2011). Meanwhile, the method precision was determined based on the relative standard deviation (RSD) with the recommended value of $\leq 20\%$ (SANCO, 2013).

Carbofuran and 3-ketocarbofuran at the concentration range of 0.01 to 1.0 mg/kg were prepared in brinjal and soil matrices. The compounds were extracted and analysed using GC-MS/MS. The retention time for carbofuran and 3-ketocarbofuran were 5.68 and 6.45 mins, respectively. The chromatograms did not show any undesirable interference and impurities as shown in Figure 4.1. Blank extract from brinjal and soil without carbofuran also did not show any interference with the targeted compounds.



Figure 4.1: Chromatogram of 1 mg/L standard solution corresponding to (a) carbofuran and (b) 3-ketocarbofuran

A field study on the dissipation of carbofuran and its metabolite in soil and brinjal was conducted at Agricultural Research Centre, Semongok, Sarawak. Table 4.1 summarizes physiochemical properties of soil of in Semongok. It is found there are no significant difference between the surface (0-15 cm) and subsoil (15-30 cm) soils.

	Semongok Soil		
	0-15 cm	15-30 cm	
pH ^a	7.9	8.0	
Conductivity (S/m) ^a	78.6	73.7	
Exchangeable base cation ^b			
$(\operatorname{cmol}(+)/\operatorname{kg})\operatorname{Ca}^{2+}$	31.54	21.27	
$(\operatorname{cmol}(+)/\mathrm{kg}) \operatorname{Mg}^{2+}$	2.77	1.92	
$(\operatorname{cmol}(+)/\operatorname{kg}) \operatorname{K}^+$	0.49	0.53	
(cmol(+)/kg) Na ⁺	0.02	0.02	
CEC °	15.19	12.31	
Percentage clay (%) ^d	34.17	34.14	
Percentage silt (%) ^d	11.02	11.01	
Percentage fine sand (%) ^d	22.97	20.16	
Percentage coarse sand (%) ^d	31.83	34.69	
Percentage carbon (%) ^e	2.3	1.7	
Percentage nitrogen (%) ^f	0.19	0.17	
Moisture content (%) ^g	13.44	9.81	
Percentage base saturation (%) ^h	249.4	182.6	

Table 4.1:Physiochemical properties of soil in Semongok

^a pH determined in 0.01 M Cacl in a 1 : 1 soil : water suspension

^bExchangeable base cations: Analysed with ICP-OES

^c CEC₇ : Cation exchange capacity determined by ammonium acetate method (pH 7) (cmol (+)/kg)

 d Mass percentage of particle size distribution determined by sieving and sedimentation (clay <2 μm , 2 μm < slit <20 μm , 20 μm < fine sand <200 μm , 200 μm < coarse sand <2000 μm)

^e Mass percentage of carbon determined by dry combustion

^f Mass percentage of nitrogen determined by Dumas combustion method

^g Moisture content was determined by oven drying and gravimetric analysis

^h Sum of exchangeable cations (Ca²⁺, Mg²⁺, Na⁺, K⁺) / CEC₇*100

According to the physicochemical values listed in Table 4.1, it can be inferred that the properties are correlated with each other. The soil pH for both surface and subsoil soils and were 7.9 and 8.0 respectively which is slightly alkaline due to high percentage of clay as compared to sandy and silt content in soil. Meanwhile, the soil organic carbon (SOC) and total nitrogen (TN) in surface soil were both higher than the subsoil soil with a difference of 0.6 and 0.2% respectively. SOC is an important component of global carbon stock and contains around twice as much carbon as the atmosphere or vegetation (Xue & An, 2018). SOC and TN are the main indicators for estimating soil quality and serve as important reservoirs for carbon and nitrogen, and understanding the distribution of total SOC and N stocks is necessary for improving soil quality (Noble et al., 2000). These findings indicate that carbon and nitrogen content in surface soil are two significant factors that affect soil fertility in vegetation cultivation system. There is, however, no substantial carbon or nitrogen relationship between crops and soil that could indicate human interference, such as ploughing and the application of various organic materials.

In surface soil, the exchangeable bases (Ca^{2+} , Mg^{2+} , K^+ , and Na^+) were found to be higher than that of the subsoil layer with Ca²⁺ being the dominant exchangeable bases in both layers. Sand, silt, clay and organic form soils. The cation exchange capacity (CEC) of a soil indicates the soil texture (Muche et al., 2015). Soils with a greater proportion of clay and organic matter have higher CEC values. The CEC value in a field is relatively stable but can alter over time with the addition of organic matter such as buying cover crops and manure (Aprile & Lorandi, 2012). CEC is a useful soil fertility measure, since it indicates the ability of the soil to provide three essential plant nutrients; calcium (Ca^{2+}), magnesium (Mg^{2+}) , sodium (Na^+) and potassium (K^+) (Aprle & Lorandi, 2012). Due to the high soil compaction, high CEC may imply high levels of clay, low permeability and internal drainage. However, CEC will also directly affect soil pH, as each time the clay particles capture cations and thus release H⁺ which acidifies soil at high concentration. Tropical soils typically have low CEC, particularly for high sandy soils and low pH. Minerals which are very common in tropical soils as aluminium, iron and manganese oxides also contribute to low CEC. (Evaluation of Cation Exchange Capacity (CEC) in Tropical Soils Using Four Different Analytical Methods).

Table 4.2 shows the recovery performance of carbofuran and 3-ketocarbofuran. The recovery performance of carbofuran is consistently more than 90% with the minimum detectable concentration established at 0.01 mg/kg (concentration below 0.01 mg/kg is unrecoverable). For 3-ketocarbofuran, the recovery remains promising for soil but for brinjal, the recoveries appears to reduce at lower concentrations, likely due to matrix effects.

Matrix effect is a change in ionization efficiency when certain compounds are present. It could be caused by non-volatile material or compounds governed by high surface activity or the flow rate of GC-MS which may affect the extent of matrix effect. (Kruve et al., 2008) Besides that, this phenomenon could also happen due to carbofuran's chemical composition. Since carbofuran and its metabolites (3-ketocarbofuran) are polar compounds (Kruve et al., 2008), the ionization efficiency of carbofuran has found to be more affected by co-eluting compounds than the ionization of less polar compounds (Otieno et al., 2010). In the present study, the recoveries obtained were in that acceptable range of 70 - 120% with mean relative standard deviation (RSD) less than 5%, in conformity to analytical method quality control and validation procedure for pesticide residue analysis (SANCO, 2013).

The carbofuran residue concentration detected was standardized with the specified MRL of various guidelines. Neither PFA (Prevention of Food Adulteration, India) nor Codex (Codex Alimentarious Commission, FAO/WHO) have established the maximum residue limits (MRLs) in brinjal. The MRL recommended by the European Commission in 2015 was 0.002 mg/kg for brinjal. The level was then set according to the LOD for the methods used and has evolved over the years. The level was stipulated at 0.02 mg/kg in 2008 and 2011 but it was reduced to 0.01 mg/kg in 2012 and further to 0.002 mg/kg in 2015 (European Commission, 2015). Meanwhile in Malaysia, the MRL of carbofuran in brinjal is specified at 0.1 mg/kg as stated in the Sixteenth Schedule of Food Act (Malaysia Food Act, 1983) and National Bureau of Agricultural Commodity and Food Standards (2014) in Thailand. In the present study, the maximum residue limit stipulated by the European Union Law at 0.01 mg/kg is adopted.

Pesticide	Fortified level	Recovery % ± RSD, % n=3		
	(mg/kg)	Brinjal	Soil	
	0.001	ND	ND	
Carbofuran	0.01	100.0 ± 4.5	92.7 ± 4.4	
	0.1	102.8 ± 3.0	91.6 ± 2.7	
	1.0	103.0 ± 5.2	93.9 ± 0.8	
	0.001	ND	ND	
3-ketocarbofuran	0.01	74.0 ± 2.7	100.0 ± 2.6	
	0.1	65.5 ± 3.4	103.8 ± 2.1	
	1.0	94.2 ± 16.0	106.4 ± 3.2	

Table 4.2:Recovery of carbofuran and 3-ketocarbofuran in brinjal and soil

ND: Non-detectable

4.2 Field Dissipation of Carbofuran in Brinjal

The temperature and precipitation throughout the field experimental period is illustrated in Figure 4.2. The tropical weather is typically hot and humid all year round with temperature ranging between 21 °C and 32 °C. The mean air surface temperatures fluctuated between 26.2 °C and 35.8 °C with the maximum recorded at 35.8 °C on Day 10 whilst the lowest at 22.8 °C on day 29. Meanwhile, the rainfall amount was recorded daily at 0800 and the average rainfall during this study was 15.30 mm. Rain event occurred almost every day

from Day 0 to Day 60 over the experimental period which is expected to contribute significantly on the dissipation and uptake of carbofuran in soil and brinjal (Appendix E).



Figure 4.2: Temperature and rainfall data over the experimental period

4.2.1 Plant Growth

The growth of brinjal was monitored over 60 days. Figure 4.3 illustrates the growth performance of brinjal treated with and without carbofuran (fruit length, root length, plant height and fruit weight). The treated brinjal observably exhibit better growth than the control plot specifically on the plant heights and fruit weights as there are statistical difference (p < 0.05) shown on its growth meanwhile no significant difference (p > 0.05) observed on the plants' fruit and root length (Appendix F).



(a) Fruit length



(b) Fruit weight



(c) Root length



(d) Plant height


4.2.2 Carbofuran and its Metabolite Residue in Brinjal

The dissipation of carbofuran and its metabolite, 3-ketocarbofuran present in fruits and leaves of brinjal was analysed by plotting the residue concentration against time as shown in Figure 4.4 and Figure 4.5. The initial concentrations of carbofuran in fruits and leaves, on Day 0 upon treatment, were 0.121 mg/kg and 0.494 mg/kg, respectively. The residues constantly increased within the first 7 days after carbofuran application. The residue concentration reached its maximum at 0.145 mg/kg and 1.897 mg/kg, respectively. After that, the concentration was seen to decrease until 60 days after treatment. Samples taken on 30 days after treatment demonstrated non-detectable carbofuran (below the detection limit of 0.01 mg/kg) for brinjal plants.

For the degradation products of carbofuran, only 3-ketocarbofuran was found at trace level. The initial average of 0.045 mg/kg and 0.142 mg/kg were detected in fruits and leaves samples, respectively. The concentration was found the highest between Day 3 and Day 7 after treatment; after 30 days, the concentration levelled at 0.05 mg/kg similar to that of the control.

Brinjals harvested from the treated plants observably grew better than the control. The weight and appearance of treated fruits were larger and heavier than the control. According to Prodhan *et al.* (2015), brinjals is an easy target to pests and disease infestation from seedling to fruiting stage. In the field, insects such as ants, fruit borer and weevil were found on the untreated fruits affecting the growth of fruits. Brinjal is subjected to infestation by various insect and non-insect pests from nursery stage to harvest. Mall et al. (1992) reported 92.5% of fruit damage and yield reduction up to 60% as a result of infestation by shoot and fruit borers - the most serious and destructive of all insect pests (Nair, 1975; Mohapatra et al., 2013).



Figure 4.4: Carbofuran residues in fruits and leaves of brinjal plants



Figure 4.5: 3-ketocarbofuran residues in fruits and leaves of brinjal plants

The residue profile of brinjal shows a moderate increase over time. It is also observed that the carbofuran residue taken up increases over time reaches its peak and follows by a consistent reduction. According to Robert and Hutson (1998), carbofuran has the lipophilicity optimum for translocation and transpiration in plants. It can be translocated from roots to shoots via the xylem vessels. Upon the degradation process, carbofuran is converted into 3-hydroxcarbofuran and subsequently to 3-ketocarbofuran. However, these metabolites are hydrolytically unstable and unlikely to be accumulated in plants (Talebi et al., 1993).

As mentioned, carbofuran is a systemic insecticide where the compound is absorbed to the plant through its roots and dispense to other parts especially the vessel, leave and stems where the insecticidal concentrations is accumulated (Otieno et al., 2011). Some studies show that the duration for carbofuran to be absorbed into plant roots is within 7 to10 days after application (Trevisan et al., 2004; Crocker, 2005) and it will eventually be broken down. A marked decline was recorded from day 7 onwards indicating uptake and degradation of carbofuran residue. The dissipation of carbofuran residue is attributed to various factors such as photodegradation, volatilization, wash-off by rain, growth dilution factor and plant metabolism (Zablotowicz et al., 2005; Franke & Juraske, 2013). No carbofuran residues were found in the brinjal fruits after 30 days indicating negligible inflow of carbofuran in its fruits and leaves.

Table 4.3 and Figure 4.6 show the regression equations for estimation of half-life of carbofuran in brinjal fruits and leaves. For the half-life in leaves and fruits, the maximum concentration from Day 3 and onwards were used to express the regression fit. The half-life of carbofuran in leaves in brinjal was predicted at 3.22 days whilst in brinjal fruits, it was

slightly longer at 10.68 days. The dissipation half-lives of brinjal fruits (10.68 days) and leaves (3.22 days) determined based on the field study are comparable to the database of carbofuran in plants reported with an average of 11.63 days in fruits (variability 11.00 to 13.10 days) and 5.03 days in leaves (variability 1.10 to 13.00 days) (Fantke & Juraske, 2013). The half-life of carbofuran fruit is comparable to half-life of benfucarb which was found to be 3.90 days and 4.73 days at 0.25 μ g/g respectively (Chandra et al., 2009). The study suggested the higher half-life could be attributed to possible slow conversion of the parent compound to its metabolites. The experimental results also suggested a pre-harvest interval of 28 days for carbofuran residue to dissipate below MRL of 0.01 mg/kg in brinjal. The main pathway of dissipation is likely hydrolysis, evaporation, photodegradation, transformation in plant and growth dilution (Fukuto, 1990; Chandra et al., 2009). It usually takes 50 to 60 days for brinjal fruits to be harvested. With a pre-harvest interval of 28 days, it is hence anticipated that the mature fruits will be safe for consumption upon harvesting.

	Regression equation	\mathbf{R}^2	Half-life, $t_{1/2}$ (day)
Brinjal fruits	Y = -0.0649 x + 1.6029	0.8815	10.68
Brinjal leaves	Y = -0.2153 x + 1.9056	0.7494	3.22

Table 4.3: Half-life $(t_{1/2})$ of cabofuran in brinjal plants and soil



Figure 4.6: The regression models for the estimation of carbofuran half-life in brinjal plants

4.2.3 Carbofuran and its Metabolite Residue in Soil

The residue (expressed in mg/kg and percentage respectively) of carbofuran in soil over the experimental period are illustrated in Figure 4.7 and 4.8. The initial concentration in soil was 2.25 mg/kg on Day 0. The level rapidly declined to 0.48 mg/kg on Day 3 (loss of 78.7%). There was a steady decrease with no detectable carbofuran after day 24 and subsequently below the limit of detection (< 0.01 mg/kg) on Day 28. Meanwhile, the concentration of 3-ketocarbofuran (as shown in Figure 4.9 was below the detection limit on Day 0 but rose to 0.081 mg/kg between Day 17 and Day 24 after application. Both the concentration of carbofuran and 3-ketocarbofuran levelled off after 30 days of treatment at 0.003 and 0.012 mg/kg, respectively.

Soil cultivated with brinjal was sampled after the brinjal plants were uprooted. As shown in the meteorological data, precipitation was experienced throughout the experimental period. The concentration of carbofuran in soil was found to consistently decrease from Day 0 to Day 60. According to Suett (1987), carbofuran could remain in the field for two to six weeks before it began to dissipate in soil. The initial lag phase however was not recorded in this study likely due to the typical tropical climatic conditions of greater rainfall and higher temperature (Chai et al., 2008) whilst the study of Suett (1987) was based on the experience of the temperate environment. It is anticipated that carbofuran will dissipate more rapidly through leaching or wash-off by rain due to its high water solubility and high mobility (Getzin, 1973; Farahani et al., 2007). Although carbofuran has a low vapour pressure, dissipation *via* volatilisation or evaporation may also took place but could be in a slower rate (Evert, 2002).



Figure 4.7: Carbofuran residues in soil of brinjal over the experimental period



Figure 4.8: Percentage of carbofuran residues in soil over the experimental period



Figure 4.9: 3-ketocarbofuran residues in soil of brinjal over the experimental period

	Regression equation	R ²	Half-life, $t_{1/2}$ (day)
Soil	Y = -0.5676 x + 0.8681	0.9982	1.22

Table 4.4:Half-life $(t_{1/2})$ of cabofuran in soil



Figure 4.10: The regression models for the estimation of carbofuran half-life in soil

Table 4.4 shows the regression equations for estimation of half-life of carbofuran in soil. The half-life of carbofuran in soil was deduced with the concentrations attained for the first 7 days. The half-life in soil from brinjal plot was predicted at 1.22 days. Rapid dissipation of carbofuran was evidenced at the onset of the field study suggesting wash off of the compound and its high aqueous solubility nature (Willis & McDowell, 1982; Wauchope et al., 2004; Chai et al., 2009; Mohamed et al., 2013). Lalah et al. (2001) stated that dissipation of pesticides happens rapidly in tropical soil where high temperature which

affects solar radiation and also wind and soil moisture retention that contribute to rapid volatilisation. Wash-off by rainfall also influenced the dissipation of pesticides if it comes immediately after pesticide application. Carbofuran has a high water solubility of 700 mg/L makes it easier to volatize from soils with high moisture content. The functional groups in carbofuran (-C=O, -COOH, and -NH) could be an influence to the degradation pathways of carbofuran which prone to affect the interaction with soil colloids, soil organic carbon or microorganisms (Hassall, 1982; Lalah et al., 2001). The loss of carbofuran under tropical climatic conditions is expected to accelerate *via* volatilization, hydrolysis (10 days), photodegradation (40 days) (Campbell et al., 2004) and wash-off by rain (1.3 weeks) (Hill and Inaba, 1991; Campbell et al., 2004; de Melo Plese et al., 2005).

Sanchez-Bayo and co-workers asserted that carbofuran is a relatively persistent insecticide; its half-life was between 23 and 46 days, falling within the range of 46 to 117 days reported by Caro et al. (1973). The half-life of carbofuran predicted in this study (1.22 days) are shorter than the half-lives reported by Chai et al. (2008) for chlorpyrifos (3.3 days) and acephate (8.7 days) based on tropical climate setting. Tropical climate is characterised with greater solar intensity, higher temperature and rainfall. A higher temperature will promote faster degradation with increased volatilization whilst higher precipitation encourages degradation through leaching and runoff (Sanchez-Bayo & Hyne, 2011).

4.3 **Response Surface Methodology (RSM)**

The dissipation of carbofuran in soil under field condition involves a few processes; these include leaching, volatilisation, wash-off from rain and degradation which rely on environmental factors (Laabs et al., 2002). These processes complicate the dissipation dynamics of pesticides. Metabolism and persistence of pesticides in plants, animals and soil have been associated with hydrolysis, hydroxylation, oxidation metabolic pathways (Murthy & Raghu, 1991). The factors that govern the degradation of carbofuran include moisture, pH, temperature, microbial population and organic matter (Evert, 2002). The effects of pH, moisture and temperature on degradation of carbofuran were evaluated based on response surface methodology (RSM). Conventionally, optimization was carried out by monitoring the influence of one parameter at a time on experimental response whilst the other parameters were kept constant. This method is an inconvenience because it does not include interactive effects among the factors studied. Moreover, the traditional method also increases the number of experiments in a research hence it consumes more time, reagents and materials (Bezerra et al., 2008). Eventually, RSM was introduced by Box and Wilson (1951) and it comes in handy because it can be applied by using multiples sets of responses that are influenced by several factors (Montgomery et al., 2012). RSM also describes the behaviour of data sets in an experiment. Therefore, in this study, RSM was carried out to investigate the optimization of degradation of carbofuran. Linear equation was best fitted for modelling of carbofuran degradation.

Moisture, pH and temperature are the three factors governing the degradation of carbofuran in soil. With RSM, these factors can be systematically optimized regarding their interacting effects. The linear, interaction and quadratic models were derived based on RSM where the magnitudes of coefficients suggest the contribution of various factors on the response. The concentration of carbofuran attained before the incubation experiments was 4.99 mg/kg. Table 4.4 tabulates the percentage of carbofuran degraded corresponding to the experimental conditions.

Experiment	Experime	Percentage of		
number (n)	X1	X2	X3	carbofuran degraded (%)
1	0	0	0	52.08
2	0	0	0	72.55
3	0	0	0	77.15
4	0	0	0	81.56
5	0	0	0	84.03
6	0	0	0	83.43
7	0	0	0	80.49
8	0	0	0	70.34
9	0	0	0	82.90
10	0	0	0	87.11
11	0	-1.68	0	50.97
12	0	1.68	0	75.42
13	-1.68	0	0	67.87
14	1.68	0	0	97.80
15	0	0	-1.68	72.01

Table 4.5:Percentage of carbofuran degraded

Experiment	Experin	nent design (Co	Percentage of	
number (n)	x1	X2	X3	carbofuran degraded (%)
16	0	0	1.68	87.11
10	0	0	1.00	07.11
17	-1	-1	-1	69.34
18	-1	1	-1	65.66
19	-1	-1	1	72.14
20	-1	1	1	71.34
21	1	-1	-1	98.26
22	1	1	-1	98.13
23	1	-1	1	97.90
24	1	1	1	92.25

Table 4.5continued

The equations below show the linear, interaction, and quadratic models with their corresponding regression coefficients where x_1 , x_2 and x_3 represents pH, moisture and temperature, respectively. It is evidenced that the temperature exerts greater influence on the dissipation of carbofuran in all models.

Linear model

$$y = 78.66 + 11.60x_1 + 2.26x_2 + 2.02x_3$$
 Equation 4.1

Interaction model:

$$y = 78.66 + 11.60x_1 + 2.26x_2 + 2.02x_3 - 0.16x_1x_2 - 1.84x_1x_3$$
 Equation 4.2

- 0.33x₂x₃

Quadratic model:

$$y = 77.00 + 11.60x_1 + 2.26x_2 - 2.02x_3 - 0.16x_1x_2 - 1.84x_1x_3$$
 Equation 4.3
- 0.33x_2x_3 + 3.678x_1^2 + 3.38x_2^2 + 2.52x_3^2

The R^2 value calculated for linear, interaction and full quadratic models are 0.489, 0.469 and 0.618 respectively. The adjusted R^2 values are 0.412, 0.318 and 0.373 implying that the degradation is more suitably described with the linear model. Table 4.5 summarizes the fitness of linear, interaction and quadratic models.

Table 4.6: The fitness of linear, interaction and quadratic models

Model	RSME	\mathbf{R}^2	Adjusted R ²	<i>p</i> -value
Linear	10.12	0.489	0.412	<i>p</i> < 0.05
Interaction	10.91	0.469	0.318	<i>p</i> > 0.05
Quadratic	10.46	0.618	0.373	<i>p</i> > 0.05



Figure 4.11: Effects of (a) pH, (b) temperature and (c) temperature against percentage of degradation

RSME serves to cumulate the magnitude of the errors in predictions into a single measure if predictive power based on the observed and predictive values. The RSME of linear, interaction and quadratic models on the degradation of carbofuran under the effects of pH, temperature and moisture are 10.1, 10.9 and 10.5, respectively. This suggests that the linear model is best fitted to describe the degradation of carbofuran in soil. The response plots of pH, moisture and temperature on the degradation of carbofuran are shown in Figure 4.11. Figure 4.11 illustrates the two-dimensional linear response surface plots of percentage of carbofuran degradation against its respective factors (pH, moisture and temperature). The surface plot of degradation percentage (%) vs pH indicates that at higher pH, the dissipation of carbofuran is favoured attaining elevated percentage of degradation. This is possibly due to the reduction of carbofuran adsorption with the decrease in adsorption site (Arias-Estévez et al., 2006). Therefore, this stipulates that hydrolysis is likely occurred in alkaline soils. The degradation of carbofuran is in addition improved with increased of soil moisture and temperature, though not as significant (p > 0.05) as compared to the soil pH. The maximum degradation of carbofuran of 98.3% is attained when the soil was adjusted to pH 1, 30%

moisture and incubated under 30 °C (coded value: +1, -1, -1 respectively) which conveys that the soil pH dominates the dissipation of carbofuran.

Table 4.7 shows the estimated regression coefficients and the corresponding *t* and *p* values; all linear, interaction and quadratic terms are characterized by p > 0.05 except x_1 suggesting a significant linear relationship between pH and degradation of carbofuran. The positive term on pH indicates that the degradation of carbofuran will accelerate as pH increases. Statistically, this proves that pH demonstrates a significant effect on the carbofuran degradation (p < 0.05) when it was subjected to rapid breakdown in more alkaline soil with the degradation percentage of 68% at pH 2 (coded value -1.68) and 98% at pH 12 (coded value 1.68). Meanwhile moisture and temperature were found to exert no significant effect on the degradation of carbofuran (p > 0.05).

Using the linear equation, the conditions anticipated with the least degradation of carbofuran was at pH 2, moisture 14% moisture and temperature of 40 °C. An experiment was carried out to verify the conditions. The soil conditions were fixed to pH 2, moisture 14% and incubated at 40 °C for 24 hours. The residue of carbofuran attained in soil was analysed in triplicates. According to the model, the percentage of degradation at the aforementioned setting is predicted at 56.11%. The experimental loss of carbofuran was recorded at 59.38% exhibiting an error of 5.82%. This implies that the model predicts the degradation of carbofuran with reasonable accuracy.

Term	Coefficient	Standard deviation	t	р
Constant	78.66	2.07	38.06	3.90×10^{-20}
x ₁	11.60	2.74	4.23	0.0004
Х ₂	2.26	2.74	0.82	0.4197
x ₃	2.02	2.74	0.74	0.4690

Table 4.7: Estimation regression coefficients and the corresponding t and p values

where x₁: pH; x₂: Moisture; x₃: Temperature

4.3.1 Effect of Soil pH

Based on the linear model, it is observed that pH gives a distinguished slope for carbofuran in soil at pH 2, 4, 7, 10 and 12. It is shown that carbofuran was subjected to rapid breakdown in a more alkaline soil (pH 8 and above) within the time required for 50% breakdown between the soils at pH 2 and pH 12. Statistically, pH demonstrates a significant effect on the degradation of carbofuran (p < 0.05)

Previous study by Siddaramappa et al., (1978) stated that carbofuran is prone to breakdown in alkaline medium where hydrolysis process predominates; the compound is subjected to slower breakdown under acidic and neutral conditions where the primary processes involved are chemical and microbial degradation. Hydrolysis occurs at the carbamate linkage yielding metabolites of 3-ketocarbofuran, carbofuran phenol and 3ketocarbofuranphenol in soil (Mora et al., 1996). A study by Arias-Estévez et al. (2006) reported a rapid adsorption of carbofuran in acidic soil which states that the adsorption of carbofuran is higher at lower pH due to increase of cationic carbofuran percentage. Lower soil pH will increase electrostatic interactions with the soil negative charge. A lower pH can also limit the dissolution of some organic matter and improve carbofuran adsorption because of the increase in adsorption site (Arias-Estévez et al., 2006). In relation to that, Ahmad and co-workers (1979) also confirmed that carbofuran is subjected to rapid chemical hydrolysis as the primary route in alkaline soils; the compound is rather persistent at a neutral pH (6.8-7.4). Likewise, Venkateswarlu et al. (1977) revealed that carbofuran degradation is unfavoured under the extreme acid conditions (Caro et al., 1973). In contrast, other carbamates such as carbosulfan and aldicarb undergo rapid hydrolysis with a decrease in pH while at higher pH (pH 6 to 8), it becomes relatively unstable (Given & Dierberg, 1985; Sahoo et al., 1993).

An investigation on microbial breakdown of carbofuran also stated that increasing pH could cause an improved rate of degradation (Read, 1986). Moreover, studies on other pesticides reported that soil with pH around 7 would have higher degradation rate over those at lower pH (Müller et al., 2007). However, a study by Vidali (2001) deduced that the sorption of prometryn on clay montmorillonite is stronger at acidic medium (pH 3) than at neutral or alkaline medium. For a given pesticide, it is vital to understand the effect of soil pH on degradation (Shahgholi & Ahangar, 2014). The soil pH is found significant to degradation rate of carbofuran in the present study, therefore, it is important to note that the soil pH plays a role in maintaining the enhanced degrading capability of carbofuran for prolonged periods.

4.3.2 Effect of Soil Moisture

The results show that the moisture level has a positive influence on the degradation of carbofuran. The soil moisture was designed at 16%, 30%, 50% and 84% according to the central composite design. The dissipation of carbofuran was evidenced to increase with increasing soil moisture with rapid breakdown at 50% moisture. More than 80% of carbofuran loss was recorded from the initial concentration. Kazemi et al. (2009) corroborates the findings of this study suggesting that carbofuran is relatively stable and persistent in dry soils with faster degradation at 50% moisture level.

Venkateswarlu et al. (1977) likewise reported accelerated degradation of carbofuran with more than 75% loss in flooded soil. This was also evidenced in organochlorine and organophosphate insecticides. The rapid decomposition of carbofuran in flooded soil is attributable to participation of anaerobic microorganisms where water serves to facilitate the pesticides movement and diffusion (Shahgoli & Ahangar, 2014). Meanwhile, Getzin (1973) and Rajagopal et al. (1984) explained that hydrolysis was bound to take place in flooded soil and produced carbofuran phenol as the main metabolite which is bound to soil components. The metabolite is susceptible to be found in flooded soil, however, in non-flooded soil, it is difficult to extract and recover the metabolite thus its finding is scarcely reported in the literature (Talebi & Walker, 1993).

Previous study by Yen et al. (1997) stated that the dissipation rate of carbofuran could increase at 100% moisture content in clayey and clayey-loamy soils. Similar observation was revealed by Gorder et al. (1982) where the degradation rate of carbofuran in cornfield soil was recorded to accelerate after precipitation. Kale et al. (2001) further supported the accelerated degradation of carbofuran in flooded soil and emphasized that moisture content and temperature were the key factors governing the the half-life of pesticides in soil (Tariq et al., 2006). A study by Lee-Yin et al. (2013) in three Malaysian soils also confirmed that the degradation of cyfluthrin was greatly affected in soil with 80% moisture than that at 30%. This findings corroborated the observation reported by Racke et al. (1994). Phorate, an organophosphate insecticide on the contrary was found to be more persistent in flooded soil than in non-flooded soil (Wardle and Parkinson, 1992).

Mojašević et al. (1996) reported faster degradation rate of carbofuran in Codorus silt loam soil at 12 to 25% moisture; the degradation was found constant at above 25%. In contrast, Benicha et al. (2013) found an increase in bound carbofuran residue with increased soil moisture. With regards to the present study, it is noted that the degradation of carbofuran varies according to soil moisture content which in turn reflecting the activity of soil microorganisms. However, in this study, autoclaved soil was used therefore microbial activity is inhibited because of the elimination of microorganisms (Benicha et al., 2013). Nonetheless, under normal soil conditions, the microbial activity is greatly affected by soil moisture content. This can be affirmed from a study on carbofuran mineralization in soil by Benicha et al. (2013). These authors found the rate of mineralization of carbofuran increases with soil moisture content which was influenced by soil microorganisms. This is comparable with a study by Shelton et al. (1991). Their study also concluded that microbial metabolism will be delayed or inhibited under soil with low moisture content or humidity meanwhile the microorganisms' activity and pesticide degradation will be generally facilitated when the soil moisture content is high (Parkin & Shelton, 1994; Mojašević et al., 1996).

4.3.3 Effect of Temperature

The influence of temperature on carbofuran degradation in soil was also taken into account. Its effect on carbofuran degradation was not as pronounced as that of moisture and pH. The degradation of carbofuran in sterilized soil was compared at 16, 30, 50, 70 and 75 °C. Throughout the experiment, it was observed that at higher temperature (50 °C and above), the soil became drier where evaporation and volatilization took place. It is evidenced that increased in temperature will enhance the rate at which carbofuran volatilizes as the moisture-evaporation factor was instigated (Souter & Burdis, 2003).

Carbofuran is known to have a relatively low vapour pressure of 8.3×10^{-6} mmHg at 25 °C (Evert, 2002), thus it is prone to hydrolysis compared to volatilization (Lalah et al., 2001). A previous study by Farahani et al. (2007) concluded that at higher temperature, the adsorption of carbofuran decreased at low concentration level; as the concentration increased, the change of temperature towards the adsorption of carbofuran was insignificant. The increase of temperature leads to increased adsorption as it is an exothermic process. The process corresponds to strengthening of attractive forces between pesticide molecules as solute and soil surface. The solubility of pesticides also plays a role in the process where the attractive forces increase with increasing water solubility (Gao et al., 2012). Similarly, high temperatures are known to increase the vapour pressures of carbofuran which in turn devour their desorption factor. Not only that, temperature elevation also causes loss of water from preferential adsorption sites in soil. This occurrence is making these sites available to carbofuran, and while in absence of active sites, the carbofuran molecules are eventually lost to the atmosphere through volatilization (Tiryaki et al., 2010). The findings in this study agreed with the observation recorded in the literature where the degradation of carbofuran was positively correlated with the soil temperature (Evert, 2002). Sahoo and co-workers (1990) observed that the disappearance rate of carbofuran is much faster at 35 °C than at 25 °C, governed by hydrolysis mechanism. It was also found that in loamy soil, carbofuran dissipated faster at 30 °C than at 10 °C (Parkin & Shelton, 1994). Meanwhile, Ramanand et al. (1998) observed that the degradation of carbofuran was only affected under temperatures higher than 25 °C. Chapman et al. (1986) reported accelerated degradation of carbofuran when the soil temperature increased to 15 °C and 35 °C.

These findings of increased degradation rate of carbofuran with increasing temperature are also observed in other pesticides. Obrigawitch et al. (1982) learned that EPTC (*S*-ethyl dipropyl thiocarbamate) insecticide degraded rapidly at 15 and 25 °C, compared to that at 5 °C. While for chlopyrifos and alachor, the influence of temperature was also found to affect greatly on their degradation rate (Walker et al., 1992; Racke et al., 1994). Lee-Yin et al. (2013) also observed a rapid breakdown of cyfluthrin in Malaysian soil due to hot and humid conditions. Therefore, it is vital to regard that temperature is one of the factors influencing the degradation of carbofuran.

4.3.4 Half-life (t_{1/2}) of Carbofuran Residue under Laboratory Setting

The half-life of carbofuran under laboratory conditions was predicted by adjusting the soil conditions to pH 2 with soil moisture of 14% and incubated under 40 °C. These conditions were selected to verify linear model. The initial carbofuran residue in soil was 4.21 mg/kg. The level rapidly declined to 1.71 mg/kg after 24 hours (loss of 59.4%). Figure 4.13 shows the percentage loss of carbofuran in soil under laboratory conditions. Meanwhile, Figure 4.14 shows the regression models for the estimation of carbofuran half-life in soil. The half-life of carbofuran after 24 hours was predicted at 24.15 hours or 1.006 days.



Figure 4.12: The percentage of carbofuran residues in soil under laboratory settings



Figure 4.13: The regression models for the estimation of carbofuran half-life in soil under laboratory setting

Most researchers reported that the measured half-life under the laboratory setting is a result of dissipative processes including chemical degradation, biodegradation, volatilisation, anaerobic degradation and photodegradation (Ahmad et al., 1979; Willis & McDowell, 1982; Laabs et al., 2002). In the present study, the soil was subjected to laboratory conditions of pH 2, 14% moisture content and 40 °C; the acidic condition offered an explanation to the longer half-life in laboratory experiment.

Getzin (1973) reported that the half-life of 8 days for carbofuran under alkaline condition at 25 °C. Meanwhile, Seiber et al. (1978) further found that the average half-lives of carbofuran in sterilized and buffered water at two different pH of 10 and 7 were 1.2 hours and 864 hours, respectively. This is comparable to the half-lives of carbaryl at 252 hours and 864 hours under similar pH of 10 and 7, respectively. These results supported that the degradation of carbofuran was mainly via the pathway of hydrolysis in alkali condition. Another investigation by Ahmad et al. (1979) reported the half-life of technical and granular carbofuran at 11 to13 days and 60 to 75 days, respectively. Moreover, Mohamed et al. (2013) did a study on the effect of temperature, moisture content and microbial activity in sterilized and non-sterilized soil. The half-life of carbofuran in soil, under high temperature and soil moisture content, ranged from 26 to 90 days for non-autoclaved soil and 39 to 142 days for autoclaved soils. The faster dissipation rate in non-autoclaved soil was attributable to the breakdown of carbofuran by microorganisms. Caro et al. (1976) and Howard (2017) also reported a longer half-lives of carbofuran, ranging from 46 to 117 days and 30 to 120 days, respectively, depending on the pH, soil moisture and temperature.

Studies of other pesticides under the effects of pH, moisture or temperature also predicted a varying range of half-life. Walker et al. (1992) mentioned the half-life of alachlor

in moist soil from 11.3 to 34.8 days and 95.9 to 279.6 days at 25 °C and 5 °C, respectively. Meanwhile, Racke et al. (1994) estimated the half-lives of chlorpyrifos in five different soils from termite-infested regions from 175 to 1576 days under standard conditions (25 ° C, field moisture capacities, darkness). A comparison on the persistence of atrazine and metolaclor insecticides also reported similar half-lives from 5.7 to 100 days and 23 to 407 days respectively under the temperature of 5 to 35 °C in field capacity (Dinelli et al., 2000). On the other hand, Singh et al. (2003) found that the half-life of chlorpyrifos was longer in acidic soil at pH 4.7 (256 days) whilst at pH 7.7 and 8.6, the degradation was quicker (16 days). When chlorpyrifos was adjusted to a more alkaline soil, the degradation half-life was relatively shorter than that in acidic soils (Singh et al., 2003)

Variation in the degradation rate of carbofuran compared between the present study and previous studies suggested the physicochemical properties of carbofuran is influenced significantly by factors such as pH, moisture and temperature.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Brinjal (*Solanum melongena*) is considered one of the important vegetable crops due to its nutritive value and it is widely planted in tropical countries such as Malaysia. However, its growth and productivity has been depleting from the attack by a number of insect pests such as shoot and fruit borer from seedling to fruiting stage. This issue is a serious concern to farmers in Sarawak as it affects the production yield thus the usage of pesticides are broaden to overcome this problem. Nevertheless, this excessive dependence on pesticides would lead to serious residual problems in crops and hence may be harmful to ecosystem, non-target organisms and human health. Therefore, to ensure food safety, farmers are advised to practice Good Agricultural Practice (GAP) and obey the recommended pesticide dosage. In addition, MRL regulations oblige a pre-harvest intervals (PHI) in order to fortify that residues level are below the proposed MRL at harvest time.

In order to protect the environment, dissipation studies on the persistence of pesticides in crops and pesticide behaviour in agricultural fields are carried out. Although carbofuran has been banned in certain countries, it is widely used in Malaysia due to its efficiency in controlling rodents and other insects. The dissipation carbofuran in vegetable and soil has been broadly studied in temperate and sub-tropical climate. However, under humid tropical climate, the study of carbofuran is still limited and it needs in-depth research to better understand its behaviour in different types of crops and soil.

In this study, the persistence and behaviour of carbofuran were observed under field and laboratory conditions. The methods used for extraction and clean-up for carbofuran residue in brinjal and soil were simple and quantitative. Under humid tropic field conditions, the residues of carbofuran in brinjal were below the LOD at 28 days until 60 days after applying the treatment. Hence, the dissipation profile of carbofuran and its metabolite postulated a pre-harvest interval of 28 days in order to comply with the MRL of 0.01 mg/kg under tropical conditions with a single application at the recommended dosage. The application of carbofuran is suitable for fruit vegetables like brinjal and hence it may be safe to consume after maturity. The average half-life of carbofuran in brinjal leaves and fruits were 3.22 and 10.33 days, respectively. Meanwhile, the half-life of carbofuran in soil was 1.24 days, shorter than the literature half-life reported primarily based on temperate conditions, suggesting accelerated photodegradation, volatilization and leaching under the hot and humid climate.

This study also performs central composite design (CCD) and response surface methodology (RSM) to determine the optimum conditions that encourage the degradation of carbofuran. RSM is an effective mathematical statistic method for establishing models, and evaluating the relative significance variables in determining the optimal conditions for desirable response. In this research, soil pH, moisture and temperature are three critical variables in controlling the dissipation of carbofuran in humid tropical soils. As a result, linear model best describes the dissipation of carbofuran with pH, moisture and temperature exerting positive effects on the loss. Carbofuran is subjected to alkaline hydrolysis and accelerated with high temperature. The soil pH establishes significant effect on the degradation which in turn indicates that chemical hydrolysis is the major pathway. The model was also corroborated experimentally with an error of 5.82 % that implies the model predicts the degradation with reasonable accuracy. To summarise, CCS and RSM are suitable in identifying the interaction of multiple factors and provide model equations in order to predict the degradation of carbofuran.

Nevertheless, more studies are needed in order to grasp better understanding on the behaviour of carbofuran on different type of vegetables and different application dosage. This is to provide vital information and guidelines for local farmers in terms of pesticide usage and to ensure the fate of the environment. Besides, carbofuran dissipation behaviour in different types of cropped soil also needs further research as vegetables cultivation in Sarawak is propagating each year. This is reasonably important to ensure sustainable agriculture is well practiced in Sarawak.

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APPENDICES

Appendix A

Carbofuran residue in brinjal

Recovery concentration (mg/kg)	Fo	rtified level (mg/	kg)	
	0.01	0.1	1.0	
Blank	0.0055	0.0000	0.0203	
1	0.0156	0.1008	1.0503	
2	0.0159	0.1064	1.0973	
3	0.0150	0.1013	0.9913	
Percentage recovery (%)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
1	101.0	100.8	103.0	
2	104.0	106.4	107.7	
3	95.0	101.3	97.1	
Average (%)	100.0	102.8	102.6	
RSD (±)	4.58	2.66	0.75	

Appendix B

Carbofuran residue in soil

Recovery concentration (mg/kg)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
Blank	0.0044	0.0511	0.0572	
1	0.0133	0.1405	0.9928	
2	0.0141	0.1422	0.9928	
3	0.0136	0.1453	0.9798	
Percentage recovery (%)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
1	89.0	89.4	94.6	
2	97.0	91.1	94.0	
3	92.2	94.2	93.2	
Average (%)	92.7	91.6	93.9	
RSD (±)	4.36	2.66	0.75	

Appendix C

3-Ketocarbofuran residue in soil

Recovery concentration (mg/kg)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
Blank	0.0000	0.0019	0.0019	
1	0.0103	0.1074	1.0908	
2	0.0099	0.1032	1.0270	
3	0.0098	0.1067	1.0787	
Percentage recovery (%)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
1	103.0	105.5	108.9	
2	99.0	101.3	102.5	
3	98.0	104.0	107.7	
Average (%)	100.0	103.87	106.4	
RSD (±)	2.65	2.17	3.19	

Appendix D

3-Ketocarbofuran residue in soil

Recovery concentration (mg/kg)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
Blank	0.0000	0.0000	0.0000	
1	0.0074	0.0681	0.7880	
2	0.0072	0.0646	0.9494	
3	0.0076	0.0639	1.0897	
Percentage recovery (%)	Fortified level (mg/kg)			
	0.01	0.1	1.0	
1	74.0	68.1	78.8	
2	72.0	64.6	94.9	
3	76.0	63.9	108.9	
Average (%)	74.0	65.5	94.2	
RSD (±)	2.70	3.43	16.0	

Appendix E

Weather meteorology

Day	Mean T (°C)	Rainfall (mm)	Day	Mean T (°C)	Rainfall (mm)
0	28.1	0.0	31	27.4	77.8
1	20.0	1.0	22	26.4	22.2
1	30.0	1.0	32	20.4	23.3
2	28.7	8.0	33	26.7	33.7
3	28.0	11.9	34	26.8	0.0
4	28.5	51.5	35	26.8	0.0
5	27.9	0.0	36	27.7	2.0
6	29.2	5.5	37	27.8	20
7	27.4	13.4	38	22.6	0.0
8	28.9	1.0	39	27.4	0.0
9	27.9	0.0	40	29.1	1.0
10	28.5	19.8	41	26.6	7.0
11	28.2	1.0	42	26.3	2.4
12	28.8	6.5	43	27.4	2.4
13	28.5	2.5	44	27.7	28.2
14	28.0	7.0	45	28.4	3.5

Day	Mean T (°C)	Rainfall (mm)	Day	Mean T (°C)	Rainfall (mm)
15	27.4	0.0	46	28.4	0.9
16	27.4	0	47	28.3	36.6
17	29.1	13.5	48	26.6	19.6
18	29.2	49.1	49	26.9	0.9
19	28.9	89.1	50	27.7	0.0
20	29.1	9.5	51	27.5	25.2
21	27.6	19.8	52	26.5	22.9
22	27.8	2.5	53	26.9	26.0
23	26.4	3.2	54	26.5	23.1
24	28.9	48.1	55	26.3	65.4
25	27.6	29.7	56	26.3	8.2
26	26.8	29.3	57	26.6	23.4
27	26.2	4.5	58	27.6	0.7
28	26.9	0	59	26.9	26.7
29	26.9	0	60	25.9	23.5
30	28.0	0.0			1

Appendix F

Brinjal plant log

Dav	Length	of fruit (cm)	Length	of root (cm)	Plant H	leight (cm)	Weight o	f fruit (g)
2.49	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0	10.5	11.1	35.3	39.3	86.7	130.0	60.6	42.3
3	11.2	8.2	27.7	32.0	118.7	125.0	62.0	60.6
7	22.7	23.0	39.3	32.7	144.3	140.0	96.5	82.6
10	25.3	24.7	35.0	35.7	148.0	166.7	115.7	123.4
14	26.3	25.3	38.0	35.3	148.7	160.3	133.6	145.8
17	27.0	31.0	35.3	39.4	156.0	164.0	174.4	152.8
21	31.3	29.3	38.0	40.7	177.7	188.7	198.7	142.0
24	30.0	28.3	37.0	40.3	196.0	211.7	169.7	144.8
28	32.0	28.0	38.3	41.7	200.0	235.0	190.4	176.1
31	27.7	26.7	38.7	38.0	191.7	210.0	231.7	181.5
38	26.7	28.1	34.5	37.8	195.7	210.3	242.1	183.0
45	28.2	27.2	39.2	37.7	192.5	213.7	228.8	188.3
60	21.3	28.3	37.0	39.7	205.7	190.0	183.7	166.8

Appendix G

	Average concentration of leaves	Average concentration of fruits
Day		
	(mg/kg)	(mg/kg)
0	0.4330	0.0320
3	1.3990	0.0420
7	1.8310	0.0560
10	0.3740	0.0090
14	0.1530	0.0070
17	0.4660	0.0190
21	0.4960	0.0560
24	0.5010	0.0410
28	0.0030	0.0200
31	0.0020	0.0001
38	0.0010	0.0002
45	0.0010	0.0004
60	0.0036	0.0030

Carbofuran residue in brinjal leaves and fruits (Brinjal plot)

Appendix H

	Average concentration of leaves	Average concentration of fruits
Day		
	(mg/kg)	(mg/kg)
0	0.142	0.042
3	0.343	0.045
7	0.416	0.056
10	0.160	0.044
14	0.130	0.046
17	0.215	0.033
21	0.185	0.016
24	0.218	0.009
28	0.093	0.039
31	0.091	0.035
38	0.091	0.035
45	0.091	0.035
60	0.092	0.035

3-Ketocarbofuran residue in brinjal leaves and fruits (Brinjal plot)

Appendix I

Carbofuran residue in soil (Brinjal plot)

	Average concentration	Percentage of carbofuran residue
Day	(mg/kg)	(%)
0	2.254	100.00%
3	0.478	21.22%
7	0.043	1.91%
10	0.119	5.27%
14	0.074	3.28%
17	0.028	1.26%
21	0.024	1.06%
24	0.023	1.03%
28	0.002	0.07%
31	0.002	0.07%
38	0.002	0.07%
45	0.004	0.18%
60	0.003	0.11%

Appendix J

3-Ketocarbofuran residue in soil (Brinjal plot)

	Average concentration
Day	(mg/kg)
0	0.015
3	0.015
7	0.015
10	0.015
14	0.049
17	0.081
21	0.063
24	0.059
28	0.012
31	0.012
38	0.012
45	0.012
60	0.012

Appendix K

	Average carbofuran residue	Percentage of carbofuran
Time (Hour)	(mg/kg)	residue (%)
0	4.208	100.0
3	2.430	57.75
6	2.274	54.05
9	2.179	51.77
24	1.708	40.60

Carbofuran residue incubated under pH 2, 14% moisture and 40 $^{\rm o}C$