UNIVERSITI PUTRA MALAYSIA

EFFECT OF GROWTH REGULATIONS (IGRS) ON THE SURVIVAL OF OIL PALM POLLINATOR WEEVIL, ELAEIDOBIUS KAMERUNICUS

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EFFECT OF INSECT GROWTH REGULATORS (IGRs) ON THE SURVIVAL OF OIL PALM POLLINATOR WEEVIL, *ELAEIDOBIUS KAMERUNICUS*

By

NUR HIDAYAH BINTI ZAILAN

A Project Report Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Sarawak Campus

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ABSTRACT

Insect Growth Regulators (IGRs) are insecticides that act on ecdysine and juvenile hormone or as chitin synthesis inhibitor. In this study, application of chitin synthesis inhibitor (flufenoxuron, lufenuron and chlorfluazuron), *Bacillus thuringiensis*, fipronil and cypermethrin may affect the percentage of weevil survival and emergence according to insecticidal effects. Thus, a laboratory study was carried out to evaluate the effect of IGRs on survival of the palm weevil *Elaeidobius kamerunicus*. Three different concentrations of each treatment were prepared using 200 mL of distilled water. Each concentration of treatment was topically applied with 1 μL of the pesticides onto the dorsum of the prothorax of adult weevil by using an eppendorf 10 μL micropipette. While dipping method was done by randomly taking three spikelets of male inflorescence at anthesis and dipped into each different concentration of treatments. Application of flufenoxuron had less hazardous effect on *E. kamerunicus* for both bioassays application as there was more than 90% weevil survival and 60% of weevil emergence by seven days after treatment (DAT). *Bacillus thuringiensis* gave more than 90% weevil survival. Lufenuron also had high weevil survival but the adult emergence was more depressed (24.14%). Chlorfluazuron had more adverse effects on percentage of weevil survival when compared to lufenuron as the chlorfluazuron was toxic but the effect was slow on adult weevil. Application of cypermethrin and fipronil were highly toxic and caused 100% mortality immediately after treatment. It was concluded that flufenoxuron and *B. thuringiensis* had high potential as alternative pesticides for pest control in the oil palm plantation as they did not harm the pollinator weevil *E. kamerunicus* and were less hazardous to the environment.

ABSTRAK

Pengawal Selia Pertumbuhan Serangga (IGRs) adalah racun serangga yang bertindak ke atas ecdysine dan hormon Juvana atau perencat sintesis kitin. Dalam kajian ini, perencat kitin sintesis (flufenoxuron, lufenuron dan chlorfluazuron), *Bacillus thuringiensis*, fipronil dan cypermethrin mungkin memberi kesan terhadap peratusan kumbang hidup dan menetas bergantung pada kesan racun. Oleh itu, kajian makmal telah dijalankan untuk menilai kesan IGRs pada keupayaan kumbang pendebungaan *Elaeidobius kamerunicus* untuk hidup dan menetas. Tiga jenis kepekatan setiap racun telah disediakan dengan menggunakan 200 mL rawatan air suling. Kaedah topikal menggunakan 1 µL bagi setiap jenis rawatan racun telah dijalankan ke atas prothorax kumbang dewasa dengan menggunakan eppendorf 10 µL micropipette. Kaedah mencelup juga dilakukan dengan mengambil secara rawak tiga spikelet yang telah berbunga dan dicelup ke dalam setiap kepekatan yang mempunyai rawatan berbeza. Kesan flufenoxuron adalah kurang berbahaya ke atas *E. kamerunicus* untuk kedua-dua bioassei kerana lebih 90% dan 60% daripada kumbang hidup dan menetas tujuh hari selepas rawatan (DAT). *Bacillus thuringiensis* memberikan lebih daripada 90% kumbang hidup. Lufenuron juga mempunyai peratusan kumbang hidup tinggi tetapi kumbang menetas adalah lebih rendah (24.14%). Chlorfluazuron memberi kesan berbahaya kepada kumbang berbanding lufenuron kerana racun chlorfluazuron bertindak perlahan ke atas kumbang hidup. Penggunaan cypermethrin dan fipronil adalah sangat toksik, menyebabkan 100% kematian serta-merta selepas rawatan. Kesimpulannya, flufenoxuron dan *B. thuringiensis* berpotensi sebagai cara alternatif untuk kawalan perosak kerana kedua-dua jenis racun ini tidak berbahaya terhadap kumbang *E. kemerunicus* dan juga terhadap alam sekitar.

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Lastly, gratitude appreciation to my beloved family, friends committee and all laboratory staffs of the Entomology Laboratory at Universiti Putra Malaysia Bintulu Sarawak Campus, for their helped and assistance.

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APPROVAL SHEET

I certify that this research project report entitled "Effect of Insect Regulators (IGRs) on the Survival of the Palm Pollinator Weevil, Elaeidobius kamerunicus" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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CHAPTER 1

INTRODUCTION

1.1 Background

Basically, to get the yield production of crops it involves some processes likes irrigation, fertilization, pollination and pruning. The interaction within environmental factors such as weather, soil, insect pest and disease problem and also management in the plantation is important as well. The management of pest is an important component for crop production. Specific pest and disease problems that attack the crops and fruits can directly reduce yield.

In oil palm plantations, the bunch moth *Tirathaba mundella* is one of the serious pests of the flowers and fruit bunches. This pest that comes from family Pyralidae is a pest that can cause fruit deformation and also bunch abortion. The infestation from bunch moth sometimes resulted from poor sanitation such as weedy field or unharvested rotten bunches on the palms (Wood 1976).

Infestation by *T. mundella* will affect oil production. Basically, to reduce the population of *T. mundella*, the planters treated infested palm with cypermethrin or fipronil (Lim *et al.* 2012). Both of these pesticides were effective in control the pest as it required a short time to kill the pest. However, these highly toxic Class II pesticides may affect the palm pollinator weevil *E. kamerunicus* and as the result may affect the yield of oil palm.

Therefore, to reduce the using of chemical pesticides which were very toxic to environment, health, food sources and non-target organisms, selected Insect Growth

Regulators (IGRs) which were Class IV insecticides were evaluated for their effect on survival of the pollinator weevil *E. kamerunicus*.

1.2 Objective

The objective of this study was to evaluate the effect of Insect Growth Regulators (IGRs) on survival of the palm pollinator weevil *E. kamerunicus*.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The problem of pest in agriculture can give big impacts especially on economic, environmental and social environment. The infection of insect pest and disease have the potential to adversely alter ecosystem function, reduce primary industry productivity and profitability and threaten human as well. Normally, to reduce and solve the number of infection, chemical insecticides were used in agriculture, household or industries.

Wojciech and Korsten (2002) reported that chemical insecticides have proven effective for increasing agriculture and forestry productivities and provide many benefits to food production and human health. However, there are some disadvantages as it can give some hazards to water and food sources, destroying of non-target flora and fauna, in food chain concentration and insect pest population could become resistant to chemical insecticides. As alternative, McDonald and Linde (2002) reported that several of biological agents formulated from bacteria, fungi, virus, plant extract and pheromones have been used to control insects responsible for forests and agriculture crops destruction.

2.2 Oil Palm

Oil palm (*Elaeis guineensis*) is a very important crop in the world as it produces vegetable oil with high saturated fat. The main product of oil palm is vegetable oil. Basically, there are two types of vegetable oil from oil palm which are crude palm oil (CPO) and palm kernel oil (PKO) (WWF 2014). One hectare of oil palm produces more than ten times the oil produced by other oil seed crops (Sime Darby 2012). The rise of oil palm industry in Malaysia was phenomenal, from less than 400 hectares planted in 1920, to 54 000 hectare in 1960 (MPOB 2012). Today, Malaysia produces 17.73 million tonnes of palm oil and 2.13 million tonnes of PKO with 5.23 million hectares of land under oil palm cultivation (MPOB 2014).

2.3 *Elaeidobius kamerunicus*, a Pollinator Weevil in Oil Palm Plantation

Elaeidobius kamerunicus in the order of Coleoptera and family Curculionidae was introduced into Malaysia in 1981 from Cameroon, Africa (Najib *et al.* 2009). The introducing of this weevil led to increase of about 36.9% to 56.0% in fruit set and 40% increase in bunch weight (Saravanan *et al.* 2014).

Before this, several species of pollinating agents were introduced but *E. kamerunicus* was the predominant species (Table 2.1) followed by a dermapteran earwig (Forficulidae), a microlepidopteran moth (Cosmopterigidae) and the Indian honey bee, *Apis cerana indica* (Hymenoptera: Apidae) (Sambathkumar and Ranjith 2011). However, all of these except for *E. kamerunicus*, did not have significance with regard to pollination in oil palm.

Table 2.1 Insect visitors on oil palm inflorescence

	Insect	Order: family	Insect visit on inflorescences	
			Male	Female
1.	African oil palm weevil (*Elaeidobius kamerunicus*)	Coleopteran: Curculionidae	/	/
2.	Earwig (*Forficula auricularia*)	Dermaptera: Forficulidae	/	/
3.	Moth (*Anatrachyntis rileyi*)	Lepidoptera: Cosmopterigidae	/	/
4.	Leaf caterpillar (*Elymnias* sp.)	Lepidoptera: Nymphalidae	/	X
5.	Citrus butterfly (*Papilio* sp.)	Lepidoptera: Papilionidae	/	X
6.	Butterfly- Yellows (*Eurema hecabe*)	Lepidoptera: Pieridae	/	X
7.	Indian honey bee (*Apis cerana indica*)	Hymenoptera: Apidae	/	/
8.	Dammer bee (*Trigina iridipennis*)	Hymenoptera: Meliponicae	/	X
9.	Black ant (*Camponotus* sp.)	Hymenoptera: Formicidae	X	/
10.	Leaf cutter bee (*Megachile* sp.)	Hymenoptera: Megachilidae	/	X
11.	Cuckoo wasp (*Thyreus caeruleopunctatus*)	Hymenoptera: Chrysididae	/	X
12.	Carpenter bee (*Xylocopa* sp.)	Hymenoptera: Xylocopedae	/	X
13.	Giant hornet (*Vespa* sp.)	Hymenoptera: Vespidae	/	X
14.	Mealy bug (*Paracoccus marginatus*)	Hymenoptera: Pseudococcidae	X	/
15.	Hover fly (*Episyrphus* sp.)	Diptera: Syrphidae	/	X

Note: (/) – Present; (X) – Absent

Franz and Valente (2005) also stated that adult weevils (*E. kamerunicus*) were highly specialised and more efficient as oil palm pollinators. These weevils were able to transfer pollen from male to female inflorescences of many adjacent palms. The weevil was dominating on both male and female flowers during anthesis of male inflorescence.

2.3.1 Development and Morphology of the Weevil

Understanding of several aspects of pollinating weevil and also male inflorescences in oil palm can help assist pollinating weevil as pollinating agents in oil palm plantation. Basri and Norman (1997) reported that about 15-30 male inflorescences were required for normal weevils for good pollination and population weevil depending to male anthesis stages. Life cycle of the weevils is totally depending on the male inflorescence on oil palm.

Tiu (2011) found that development of weevil from egg to larvae was between 2 or 3 days, and took between 5 to 10 days to become pupa. Pupa period occur within 2 to 6 days, and 18 days for the adult weevil. Sholehana (2010) reported that the life cycle of *E. kamerunicus* was 33 days that includes about 2.5 days of egg, 12 days of larvae, 5 days of pupa and 18 days for female adult weevil while 15 days for male adult weevil. She also stated that *E. kamerunicus* had a fecundity of 27 eggs in a life cycle and about 72.9% of mortality from egg to adult. The laying of *E. kamerunicus* egg varied from 35 to 48 eggs. Normally, not all the weevils could complete the life cycle as there were many limiting factors that contribute such as climate, temperature, humidity and other.

Oil palm pollinator (*E. kamerunicus*) is a tiny weevil with the size 4 mm for male and about 3 mm for female (Howard *et al.* 2001). Based on Table 2.2, Herlinda *et al.* (2006) also stated that *E. kamerunicus* is classified as a tiny weevil with size of 3.5-4 mm for adult, 3 mm for pupa, 1-3.5 mm for larvae and 0.5 mm for egg stage. The colour of adult weevil is dark brownish in head and shining brown in body. This weevil often had 3 pairs of legs, antenna, 2 pairs of wings and snout.

Table 2.2 The morphology of weevil *E. kamerunicus*

Stages	Length (mm)	Width (mm)	Morphology	Colour
Egg	0.5	0.5	Round shape	Shining yellow.
Larvae	1-3.5	1	Have head, and mouth to chew	Yellow white and at the end become yellow
Pupa	3	1.5	Have head, thorax and abdomen	Yellow and after that, it become brownish to black
Imago	3.5-4	1.2-1.5	3 pairs of legs, antenna, 2 pairs of wings, snout.	Dark brownish in head and shining brown in body.

2.4 The Pest that Attacks Fruit Bunches of Oil Palm

Lim (2012) reported that *T. mundella* was the bunch moth that had become one of the most important pests in oil palm planted on peat soil. About 50% of crop would be lost and free fatty acid (FFA) content in palm oil also increased in infested bunches if the infestation by *T. mundella* were not properly managed by the planters. *Tirathaba mundella* have a short life cycle and thus can spread fast especially with poor sanitation and in the presence of unharvested rotten bunches on the palms. If the caterpillar infested a palm, the development of female inflorescences and bunches at various stages would be attacked. Symptoms of infestation by *T. mundella* could be seen as the fruit bunches were covered with faeces and have non-glossy appearance compared to healthy bunches that have shiny appearance. Besides, on the outer layer of ripening fruits holes were seen that were caused by caterpillar feeding on them. Ariffin and Basri (2000) stated that the damaged fruits fell to the ground prematurely or developed without kernels. The eggs of bunch moth can be found from over ripe rotten bunches or in bunches or inflorescences which have fallen to the ground

2.4.1 The Morphology of Pest *T. mundella*

Forestry Compendium (2012) reported that there are always confusion to identify between *T. mundella* and *Tirathaba rufivena*. *Tirathaba mundella* is a common pest on *Elaeis* but *T. rufivena* fed on *Cocos* although sometimes it may also occur on *Elaeis*. *Tirathaba mundella* can be differentiated from *T. rufivena* by wingspan and characters on the forewing of adult moths. *Tirathaba mundella* has a wingspan of 19-22 mm and grey colour of forewing with black scales. *Tirathaba rufivena* has a wingspan of 29-34 mm and forewings reddish in colour.

The distribution of *T. mundella* mainly in Malaysia, India and Indonesia based on available information (Table 2.3). Malaysia was the widespread of this pest distribution compared to others countries (Forestry Compendium 2012).

Table 2.3 Distribution of *T. mundella* in South East Asia based on available information

Asian Country	Distribution	References
India	Present	
Andaman and Nicobar Island		Bhumannavar and Jacob (1990)
Karnata Bengalka		Daniel and Kumar (1969)
Kerala		Nair and Rawther (1969)
West Bengal		Sit and Avrajyoti Ghosh (2005)
Indonesia	Present	
Java		Waterhouse (1993); Kalshoven (1950)
Sumatra		Kalshoven and LGE; Lann PA van der
Malaysia	Widespread	Wood and Ng. (1974); Waterhouse (1993)

2.5 Types of Pesticides Normally Used in Oil Palm Plantation

2.5.1 Gram Positive, *B. thuringiensis*

Bacillus thuringiensis is a Gram-positive bacterium that produces protein crystal that can be toxic to certain insect pests (Najib *et al.* 2012). *Bacillus thuringiensis* proteins crystal are considered to be safe to human and non-target organisms such as wildlife and thus the formulation can be used essentially for all food crops (Cranshaw 2012). He also stated that *B. thuringiensis* causes bacterial disease that naturally exists in insects, and in some insecticides, it can be as an active ingredient. Several products of *B. thuringiensis* have been developed in large quantity because of the safety to user, suitable for insect pest controls and also widespread use as microbial insecticide. In global biopesticides market, biopesticides based on *B. thuringiensis* have contributed up to 90% and have been considered as valuable products worldwide compared to others insecticides (NPTN 2004) with estimated annual sales of as $100 million (Federici *et al.* 2006; Kaur 2002).

The active ingredients in *B. thuringiensis* products are a mixture of spores and crystal toxins proteins produced during the aerobic lag phase of the growth cycle of the bacteria (NPTN 2004). Ghribi *et al.* (2006) reported that these mixtures were easily harvested and formulated. The δ-endotoxins protein is solubilized in the midgut when the insect ingested the products (Ammouneh *et al.* 2010). In the presence of the specific receptor in the midgut epithelium, the crystal toxicity to the insect is determined (Bravo *et al.* 2007). Ramlah (2000) reported that several δ-endotoxins have receptor proteins in the gut lining of insects, such as the oil palm bagworms, *Metisa plana*.

Numerous research studies have been conducted on different strains of *B. thuringiensis* that affect larvae from Lepidoptera, Diptera and Coleoptera (NPTN 2004). Najib *et al.* (2009) reported that *B. thuringiensis* is very toxic to certain Lepidoptera and sometimes to non-target species. This biopesticides are toxic to small invertebrates and larvae of susceptible insects (Federici 2005; Praca *et al.* 2004). For example, it is commonly used for some leaf and nettle feeding caterpillars and also can affect fly larvae (Cranshaw 2012). During handling of *T. mundella* on fruit bunches in oil palm plantation, the speedy application of *B. thuringiensis* and good sanitation is an effective way to avoid outbreak situation (Lim 2012). Biopesticides that have *B. thuringiensis* as a based are not toxic to *E. kamerunicus*, the oil palm pollinator weevil and also other beneficial insect (Najib *et al.* 2012).

2.5.2 Cypermethrin

Cypermethrin is a Class II pesticide that used to control insects in the household and agriculture fields. It is widely used in agriculture and veterinary medicine and therefore it is considered as safe to environment (Shukla *et al.* 2002). However, uncontrolled and repetitive applications lead to unintend effects in non-target organisms. Gowland (2002) reported that cypermethrin is highly toxic to fish population and aquatic invertebrates. Slow metabolism and elimination of these compounds can relatively cause sensitivity to fish (David *et al.* 2003).

In oil palm plantation, cypermethrin is used to control leaf-eating pests such as bagworms, nettle caterpillars and rhinoceros beetles (Halimah *et al.* 2009). Najib *et al.* (2009) reported that cypermethrin was significantly toxic to *E. kamerunicus* as it causing 100% mortality even one day after treatment (DAT). Lim (2012) reported that after spraying of cypermethrin, the population of pollinating weevils, *E. kamerunicus* and earwigs, *Chelisoches moris* which is a beneficial insect and useful as predator of the *T. mundella* were affected after several observations.

Anand *et al.* (2012) reported that cypermethrin can cross the blood brain barrier and induce neurotoxicity and motor deficits. Cypermethrin can increase duration of opening of sodium channel, a major site of its action, leading to hyper-excitation of the central nervous system. In sodium channel also cypermethrin can modulates chloride, voltage-gated calcium and potassium channels, alters the activity of glutamate and acetylcholine receptors and adenosine triphosphates and induces DNA damage and oxidative stress in the neuronal cells. Cypermethrin also can modulate the level of neurotransmitter that including gamma-aminobutyric acid (GABA) and dopamine.

2.5.3 Fipronil

NPIC (2009) reported that fipronil was first introduced in the U.S. in 1996 for indoor pest and commercial turf products. Fipronil is a broad spectrum insecticide of Class II toxicity that belongs to the phenylpyrazole chemical family. Mostly, fipronil is used to control beetles, ants, thrips, cockroaches, fleas, weevils and other insects. It is highly toxic to fish and aquatic invertebrates, bees, upland game birds and partially toxic to waterfowl but non-toxic to mallard ducks and other bird species.

In oil palm plantation, the common method used to control termites is by using fipronil. Fipronil is widely used for termite management (Zulkifli 2007). Lim and Silek (2001) reported that fipronil is good for termite control in field trials on 2.5 years oil palms. From the symptoms that observed and severity of attack, three stages of infestation are classified which are initial, intermediate and advanced stage. Therefore, after fipronil treatment was applied, there was 100% recovery of palms when treated at the initial stage of infestation, about 90% at intermediate stage of infestation and 20% for advanced stage of infestation on palms.

Fipronil is effective for insect by ingestion and causes neutral excitation and convulsion in insects at the end resulting death for insects (FAO 2009; NPTN 1997). Fipronil kills the insects when they eat it or in contact with it. It works by disrupting the normal function of the central nervous system in insects.

At normal room temperature, fipronil can be stable for one year in the absence of metallic ions. Fipronil and some of its degraders may bioaccumulation, particularly in fish (Tingle *et al.* 2003). Fipronil is not readily considered for biodegradable (NPTN 1997). Lack of oxygen in water and sediment can slowly degrades the fipronil pesticides with a half-life of 116-130 days. It is adsorbed to suspended solid and sediment. In basic solution, the half-life is 28 days and it remains stable to breakdown by water at a mildly acidic to neutral pH. Fipronil has a half-life of 3.6 hours in water when exposed to sunlight and about 3 to 7 months half-life on vegetation.

2.6 Insect Growth Regulators (IGRs) Class IV Pesticides

David (2002) reported that Insect Growth Regulators (IGRs) disturbed embryonic, larval and nymph development, and disrupts metamorphosis and reproduction. It is highly selective to insects and arthropods. IGRs will prevent insects from maturing into adults and have low environmental toxicity with reduced risk to non-target insects.

IGRs can be separated into three categories based on mode of action which are juvenile hormone (JH), ecdysone and chitin synthesis inhibitors (CSI) (Hoffman and Lorenz 1998). Based on these categories, JH arrest development and cause insects to remain in immature stage, preventing them from completing their life cycle. Ecdysone disrupts molting by inhibiting metabolism of the molting hormone and CSI interfere with enzymes that stimulate the synthesis and formation of chitin which is an essential component of the exoskeleton. Without chitin, insects die in immature stage or mature into sterile male or female adults (Cloyd 2013).

James (2002) claimed that the hormones are indispensable to insect development and are involved in other important processes such as spermatogenesis, vitellogenesis, oogenesis and sexual behaviour. Ruberson *et al.* (1998) reported that the effects of IGRs were to disturb development and metamorphosis and action in insect pest. Therefore, these compounds can be said were safe to natural enemies and alternative ways to replace chemical insecticides because lethal and sub-lethal effects of the latter are considered highly risky to beneficial species.

2.6.1 Lufenuron

Lufenuron is a benzoylphenylurea class of insecticide which act as a chitin synthesis inhibitor. It has been proven in the treatment of fleas feeding on dogs and cats by oral or parenteral administration (Dean *et al.* 1999; Meola *et al.* 1998). The high dosage of lufenuron recommended for anti-flea treatment was very effective in treating dermatomycoses in dogs and cats (Ben-Ziony and Arzi 2000). It was having high demand due to their low mammalian toxicity and no mutagenic and teratogenic effects on warm-blooded animals (Mondal and Parween 2000).

Besides, lufenuron also provides for control of larvae of some insect leaf miners and fruit flies (Diptera), psyllids and flocculent white fly (Homoptera) and rust mites. Lufenuron can be used in a wide range of crops such as cotton, maize, sugar beet, potatoes, other vegetables, grapes, citrus, other fruit, and ornamentals (Deivanayagam *et al.* 2010).

Lufenuron is only active against immature larval and molted nymph stage of arthropods. It is active in digestion activity. Optimum control of fruit pests is obtained when lufenuron is applied at egg hatching. Lufenuron could not cause pupa mortality, irrespectively of the pupa stage. It exerted some action on adult morphogenesis (Ghoneim *et al.* 2003). Lufenuron was effective as larvicide against 8 to 20 days old larvae but had little effect on newly hatched larvae of Lepidoptera (Charmillot *et al.* 1991).

The residues of lufenuron are unlikely to occur in crops planted after crop harvested as demonstrated by crop rotation. Lufenuron is unlikely to be taken by plants as it is lipophilic. Soil contamination is only possible issue but it can easily be removed during preparation of food before consumption (EFSA Scientific Report 2008).

2.6.2 Flufenoxuron

Flufenoxuron is benzophenyl urea insecticide or acaricide developed by Shell Research Ltd. (United Kingdom) which inhibits chitin biosynthesis (MOA Group 15) in nymphal mites and Lepidoptera larvae. Flufenoxuron is formulated as 50 and 100g/L EC, DC or FIC under trade name Cascade. These formulations are depending on the crop and the country of use that proposed for one to four foliar applications per season to the above crops at rate equivalent to 40-250 g a.i/ha/application.

Kondo *et al.* (2002) reported that flufenoxuron is chitin synthesis inhibitor that interferes with the exoskeleton chitin by contact due to its lipophilic properties. High concentration of flufenoxuron can caused anti-feeding effect. Clarke and Jewess (1990) reported that the flufenoxuron sub-lethal effects persist for a long time in insect body after ingestion due to its delayed metabolism.

2.6.3 Chlorfluazuron

Chlorfluazuron is a benzylphenyl urea (BPU) and chitin synthesis inhibitor (CSI) (Perveen 2005). It is a promising insecticide which is highly effective on Lepidoptera and Coleoptera larvae (Adel 2008).

This IGR have long life in insect bodies with a slow metabolism and elimination rate strategies. Chlorfluazuron can be adopted in pest control strategies and can be used in integrated pest management (IPM) programme which can better as pest control (Sammour *et al.* 2008; Fahmy and Miyata 1998).

CHAPTER 3

METHODOLOGY

3.1 Location and Duration of Study

The study was conducted at Entomology Laboratory of Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB). The study materials were obtained from the UPMKB Oil Palm Estate. Age of the palms ranged from 15 to 25 years old. The period of this study covered 5 months from October 2013 until February 2014.

3.2 Sources of *E. kamerunicus*

The adult pollinating weevils were collected from male inflorescences of oil palm at UPMKB. The larvae stage of the weevil was obtained from male inflorescences three days after anthesis at the flowers.

3.3 Preparation of Artificial Diet for Weevils

The artificial diets for weevils *E. kamerunicus* were prepared in the laboratory (modified from Aziza and Zamzam 2013). The lists of ingredients as given in Table 3.1. All the ingredients are blended with 1 L of distilled water except agar, vitamin B complex, vitamin C and penicillin. The agar was dissolved in another 500 mL of boiling water. After that, the agar was added and mixed with the blended mixed diet. The ingredients were autoclaved, after cooling to 45°C, vitamin B complex, vitamin C and penicillin were added to the diet and gently swirled to dissolve. The mixture was poured into Petri dish at 15-20 mL per dish, and later keeps in refrigerator until use.

Table 3.1 Ingredient of semi-artificial diets used for oil palm weevil, *E. kamerunicus*

Ingredients	Quantity
Carrot	25 g
Sugar beet (beta vulgaris)	25 g
Soya bean powder	50 g
Sugarcane	10 g
Egg yolk	1 egg
Dry milk	10 g
Brewer yeast	15 g
Sodium benzoate	2 g
Molasses	5 mL
Coconut oil	1 mL
Folic acid	2 g
Vitamin B complex	1 tablet
Agar	20 g
Ascorbic acid (vitamin C)	2 g
Penicillin	0.3 g
Distilled water	1500 mL

Note: Modification was made by removing the sorbic acid and powder of dry beans in the diet. Penicillin was added in media as antibiotic.

3.4 The Amount of Active Ingredient (a.i) that Used for Each Concentration of Treatment

The various concentrations of the treatments were prepared by using the label recommended rate, with one level at 50% below and one level at 50% above the recommended rate (Table 3.2), and the volume marked up until 200 mL with distilled water.

Table 3.2 Treatment concentrations according to active ingredient (a.i)

Chemical Names	50% above recommended rate (mg a.i L^{-1})	Recommended rate (mg a.i L^{-1})	50% below recommended rate (mg a.i L^{-1})
Flufenoxuron	270	180	90
Lufenuron	120	80	40
Chlorfluazuron	220	330	330
Bacillus thuringiensis	510	340	170
Fipronil	300	200	100
Cypermethrin	300	200	100

3.5 Bioassay Method

The IGRs flufenoxuron, lufenuron and chlorfluazuron were used as test pesticides while fipronil, *B. thuringiensis* and cypermethrin were included as common used in plantation pest control *T. mundella*, and distilled water was used as control.

3.5.1 Topical Method Application

The adult weevils were transferred into a Petri dish at 10 weevils per Petri dish. Artificial diets were placed in the Petri dish as a food source for the weevils. As an additional in food source, 10% honey was used and soaked in cotton wool in a separate small dish.

For the bioassay, each adult weevil was treated with 1.0 µL of the treatment liquid by topical application onto the dorsum of the prothorax using an eppendorf 10 µL mircropipette. Each treatment was replicated three times with 10 weevils per replicate. The weevils were incubated in the laboratory at a temperature 27°C and 50% RH. The survival of weevils was recorded daily after treatment. The Completely Randomized Design (CRD) was adopted in this experiment.

3.5.2 Dipping Method Application

Three spikelets of male inflorescence at anthesis stage were randomly taken and dipped into each concentration of pesticides without dried (Table 3.2). Each treatment was replicated for four times. The treated spikelet was transferred into a small container and incubated in the laboratory at 27°C and 50% RH. The number of adult emerged from the spikelet were recorded at seven days after treatment (DAT). The treatments were arranged in a Randomized Complete Block Design (RCBD).

3.6 Data Analysis

Data on mortality were corrected by using Schneider-Orelli formula. The data were then subjected to Analysis of Variance (ANOVA) by using SAS 9.2 software. When F value was significant, the mean were compared using Tukey's test. Schneider-Orelli formula as shown in below:

$$\text{Corrected value} = \frac{(\%\ \text{responded} - \%\ \text{responded in control}) \times 100}{(100 - \%\ \text{responded in control})}$$

CHAPTER 4

RESULTS

4.1 Effect of Different Treatments of Pesticides towards Survival of the Palm Weevil Adult (*E. kamerunicus*), by Topical Application

At 0 DAT, 100% of the weevils survived in treatment with flufenoxuron, lufenuron, chlorfluazuron and *B. thuringiensis* across all concentrations as shown in Figure 4.1. The survival trend decreased with increasing DAT. Although there was a slight decrease in survival beginning 2 and 3 DAT for flufenoxuron and lufenuron respectively, there was still more than 90% survival by 7 DAT. Treatment with *B. thuringiensis* and chlorfluazuron, percentage survival decreased as early as 1 DAT, and dropped to 87.78% by 5 DAT after which *B. thuringiensis* weevils continued to survive well while that of chlorfluazuron decreased to 66.67% by 7 DAT. Application of fipronil and cypermethrin, caused 100% mortality as early as 0 DAT which shows that the chemical was highly toxic to the weevils.

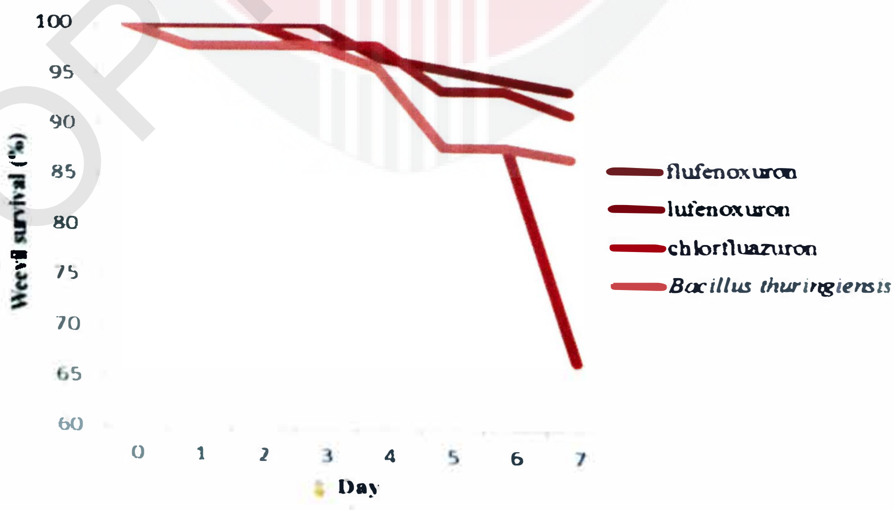

Figure 4.1 Overall daily survival of weevil across all concentrations of selected pesticides

4.1.1 Effect of 50% Increased Concentration for Each Different Insecticide on Oil Palm Pollinator Weevil, *E. kamerunicus* on 3 and 6 DAT

Increased 50% of active ingredient from standard recommended dosage in each treatment (Table 4.1) showed the high weevil survival rate for all the biopesticides with chlorfluazuron and lufenuron having the highest survival rate of 96.67% at 3 DAT. However, the survival rate dropped slightly to 83.33% at 6 DAT for both of the treatments. The survival of weevils treated with flufenoxuron was 93.33% and it maintained till 6 DAT. *Bacillus thuringiensis* had a survival rate of 93.33% at 3 DAT and reduced to 86.67% at 6 DAT. No weevil survival with treatments of fipronil and cypermethrin at both 3 DAT and 6 DAT.

Table 4.1 Survival of *E. kamerunicus* treated with 50% increase in active ingredient of recommended dosage of selected pesticides

Treatment	Concentration (mg a.i L^{-1})	n	Weevil survival (%)	
			DAT 3	DAT 6
Flufenoxuron	270	10	$93.33^{a}\pm3.33$	$93.33^{a}\pm3.33$
Lufenuron	120	10	$96.67^{a}\pm3.33$	$83.33^{a}\pm3.33$
Chlorfluazuron	220	10	$96.67^{a}\pm3.33$	$83.33^{a}\pm6.67$
Bacillus thuringiensis	510	10	$93.33^{a}\pm6.67$	$86.67^{a}\pm3.33$
Fipronil	300	10	$0.00^{b}\pm0.00$	$0.00^{b}\pm0.00$
Cypermethrin	300	10	$0.00^{b}\pm0.00$	$0.00^{b}\pm0.00$

Means with different letters indicated significant differences within column at $P\leq0.05$

4.1.2 Effect of Recommended Concentration for Each Different Insecticide on Oil Palm Pollinator Weevil, *E. kamerunicus* on 3 and 6 DAT

At the recommended dosage for topical bioassay on weevil survival (Table 4.2), treatment *B. thuringiensis* had 100% survival at 3 DAT but decreased to 90% at 6 DAT. The IGRs (flufenoxuron, chlorfluazuron and lufenuron) showed high survivability of the weevil with 96.67% at 3 DAT. Apart from lufenuron in which the survival rate was maintained at 96.67%, the weevil survival of the other two IGRs dropped to 93.33% at 6 DAT. There was no survival of weevil in treatments with fipronil and cypermethrin.

Table 4.2 The survival of *E. kamerunicus* at treatment with recommended dosage of each pesticide

Treatment	Concentration (mg a.i L^{-1})	n	Weevil survival (%)	
			DAT 3	DAT 6
Flufenoxuron	180	10	$96.67^{a}\pm3.33$	$93.33^{a}\pm3.33$
Lufenuron	80	10	$96.67^{a}\pm3.33$	$96.67^{a}\pm3.33$
Chlorfluazuron	330	10	$96.67^{a}\pm3.33$	$93.33^{a}\pm3.33$
Bacillus thuringiensis	340	10	$100.00^{a}\pm0.00$	$90.00^{a}\pm5.77$
Fipronil	200	10	$0.00^{b}\pm0.00$	$0.00^{b}\pm0.00$
Cypermethrin	200	10	$0.00^{b}\pm0.00$	$0.00^{b}\pm0.00$

Means with different letters indicated significant differences within column at $P\leq0.05$

4.1.3 Effect of 50% Reduction of Concentration from the Recommended Dosage of Different Insecticides on Oil Palm Pollinator Weevil, *E. kamerunicus* at 3 and 6 DAT

Table 4.3 showed the result of treatment by 50% reduction of active ingredient from recommended dosage by using topical application method. At 3 DAT, flufenoxuron, lufenuron and chlorfluazuron showed 100% survivability and only lufenuron remained at 100% survival rate at 6 DAT. The survival rate of *B. thuringiensis* was 96.67% at both 3 DAT and 6 DAT. For treatments of cypermethrin and fipronil, there was no survival.

Table 4.3 Weevil survival at 50% reduction of active ingredient from recommended dosage

Treatment	Concentration (mg a.i L^{-1})	n	Weevil survival (%)	
			DAT 3	DAT 6
Flufenoxuron	90	10	100.00^{a}±0.00	96.67^{a}±3.33
Lufenuron	40	10	100.00^{a}±0.00	100.00^{a}±0.00
Chlorfluazuron	330	10	100.00^{a}±0.00	90.00^{a}±10.00
Bacillus thuringiensis	170	10	96.67^{a}±3.33	96.67^{a}±3.33
Fipronil	100	10	0.00^{b}±0.00	0.00^{b}±0.00
Cypermethrin	100	10	0.00^{b}±0.00	0.00^{b}±0.00

Means with different letters indicated significant differences within column at $P \leq 0.05$

4.2 Effect of Different Treatments towards Adult Weevil Emergence by Using Spikelet Dipping Application

4.2.1 Effect of 50% Increased Concentration over Recommended Dosage for Each Insecticide on Emergence of Adult Weevil

For dipping method, the highest percentage of adult weevil emerged after 7 DAT was flufenoxuron at 42.87% (Table 4.4). This was followed by chlorfluazuron with 42.39%, *B. thuringiensis* with 34.98% and lufenuron with 17.25%. There was no adult emergence with treatments of cypermethrin and fipronil.

Table 4.4 Effect of increasing each treatment with 50% of active ingredient from recommended dosage on weevil adult emergence

Treatment	Concentration (mg a.i L^{-1})	Weevil emergence (%)
Flufenoxuron	270	42.87^{a}±2.71
Lufenuron	120	17.25^{b}±3.05
Chlorfluazuron	220	42.39^{a}±1.28
Bacillus thuringiensis	510	34.98^{a}±5.24
Fipronil	300	0.00^{c}±0.00
Cypermethrin	300	0.00^{c}±0.00

Means with different letters indicated significant differences within column at $P \leq 0.05$

4.2.2 Effect of Label Recommended Concentration of Insecticide on Emergence of Oil Palm Pollinator Weevil, *E. kamerunicus*

At the recommended dosage, emergence of adults from the spikelet was significantly higher with flufenoxuron (74.39%) followed by chlorfluazuron (64.53%) and *B. thuringiensis* (54.19%) compared to lufenuron (26.6%) (Table 4.5). The lowest was treatments with cypermethrin and fipronil where there was no emergence.

Table 4.5 Emergence of adult *E. kamerunicus* at label recommended dosage of pesticides

Treatment	Concentration (mg a.i L^{-1})	Weevil emergence (%)
Flufenoxuron	180	$74.39^{a}\pm6.09$
Lufenuron	120	$26.60^{b}\pm1.89$
Chlorfluazuron	330	$64.53^{a}\pm2.05$
Bacillus thuringiensis	340	$54.19^{a}\pm10.71$
Fipronil	200	$0.00^{c}\pm0.00$
Cypermethrin	200	$0.00^{c}\pm0.00$

Means with different letters indicated significant differences within column at $P\leq0.05$

4.2.3 Effect of 50% Reduction in Concentration on Recommended Dosage of Insecticide on Emergence of Oil Palm Pollinator Weevil, *E. kamerunicus*

Flufenoxuron had the highest percentage emergence of weevil at 78.81% (Table 4.6) followed by *B. thuringiensis* (73.40%), chlorfluazuron (55.17%) and lufenuron (28.58%). No adult emerged from treatment with cypermethrin and fipronil.

Table 4.6 Emergence of adult weevil from treatment with 50% reduction of active ingredient from recommended dosage of pesticides

Treatment	Concentration (mg a.i L^{-1})	Weevil emergence (%)
Flufenoxuron	90	$78.81^{a}\pm6.14$
Lufenuron	40	$28.56^{c}\pm1.71$
Chlorofluazuron	330	$55.17^{b}\pm1.39$
Bacillus thuringiensis	170	$73.40^{ab}\pm7.81$
Fipronil	100	$0.00^{d}\pm0.00$
Cypermethrin	100	$0.00^{d}\pm0.00$

Means with different letters indicated significant differences within column at $P\leq0.05$

CHAPTER 5

DISCUSSION

From this study, cypermethrin and fipronil were confirmed to have significant effects on the oil palm pollinating weevil (*E. kamerunicus*) adults and larvae by causing 100% mortality for all concentration levels treated. Najib *et al.* (2012) reported that cypermethrin led to 100% mortality even at 0 DAT to oil palm pollinating weevil as this insecticide has an adverse effect and highly toxic to the weevil. Cypermethrin is a Class II insecticide that kills the insects by contact. This insecticide caused the sodium channel to remain open for prolonged period compared to the normal few millisecond periods, leading to paralysis (Rodriguez *et al.* 2009). Fipronil also is a Class II insecticide that is broad spectrum and highly toxic to bees, lizards and gallinaceous birds but showed less toxicity to waterfowl (Tingle *et al.* 2003). Fipronil can affect non-target insects in Coleoptera, Hymenoptera and Diptera order (Balança and del Visscher 1997).

The effects of IGRs (flufenoxuron, chlorfluazuron, and lufenuron) were much less hazardous compared to the chemical insecticides like cypermetrin and fipronil. IGRs are important in agriculture as they have low toxicity and safer in term of food production. Michelle (2003) reported that the compounds of IGRs generally affect only animals that molt. Thus, it basically does not affect non-target species such as fish, birds, mammals or other invertebrates. Usually, IGRs kill insects during the larval and nymphal stage and in some cases during pupa stages. Similar observation occurred in this study when treatment lufenuron was applied. The result showed that treatment lufenuron gave the lowest percentage on weevil emergence (24.14%) compared to other selected pesticides. Bakr *et al.* (2008) reported that lufenuron is only active against immature larval and nymph stage of arthropods. This pesticide does not have a contact activity but more of targeting the digestion activity. After ingestion and feeding, death follows within a few days. Therefore, lufenuron is most suitable to be applied during egg hatching for pest control management but not on maintaining the survival of oil palm pollinator weevil.

Chlorfluazuron had more adverse effect on weevil when compared to lufenuron. Based on the result, treatment with chlorfluazuron was toxic but the effect was slow on adult weevil. Balanca and de Visscher (1997) reported that chlorfluazuron can metabolize slowly inside the insect body and thus only become toxic after several days. However, there was more than 50% emergence of adult weevil after treatment with chlorfluazuron. Chlorfluazuron is a promising insecticide which is highly effective on Lepidoptera and Coleoptera larvae (Adel 2008). Since pollinating weevil is a member of Coleoptera, chlorfluazuron can pose a threat to them while this insecticide is being used to control bunch moth in oil palm plantation. Therefore, usage for this chemical to control bunch month should not be above the recommended dosage (330 mg a.i L^{-1}) in order to maintain the population of the weevil.

Besides, this study revealed that flufenoxuron had very little hazardous effect on the weevils in both the adult and larvae stage as flufenoxuron had the highest percentage of the weevil survival and adult emergence. The effect is only severe in the case of the larvae when the amount of the insecticide was much higher (270 mg a.i L^{-1}) than the recommended dosage (180 mg a.i L^{-1}). This may be due to anti-feeding effect at higher concentrations of flufenoxuron (Reda *et al.* 2010).

The effect of *B. thuringiensis* against pollinating weevil was very similar to the Insect Growth Regulator (IGRs) as there was no significance difference in the adult mortality and emergence after being treated. This treatment was less hazardous to the weevil for both methods and comparable to flufenoxuron. Williams (2000) reported that *B. thuringiensis*, a biological agent, are non-toxic to human, other mammals and non-target organism. *Bacillus thuringiensis* acts upon insects as a stomach poison via the ingestion of bacteria by the insects. The amount of *B. thuringiensis* ingested by the adult weevils via topical application may be less compared to the larvae as there may be some pesticide residue on the spikelet. Hellmich and Hellmich (2012) reported that corn rootworm larvae especially larvae of the western corn rootworm can be killed by *B. thuringiensis* maize. However, the effect of *B. thuringiensis* towards the weevil is safer when compared with IGRs chlorfluazuron and lufenuron according to the percentage of adult weevil survivability and emergence.

CHAPTER 6

CONCLUSION

Use of different treatments and concentrations of Insect Growth Regulators such as flufenoxuron, lufenuron and chlorfluazuron, and *B. thuringiensis*, cypermethrin and fipronil had different effects on the percentage of weevil survival and emergence. Flufenoxuron and *B. thuringiensis* had potential to be used for control in oil palm plantation as it gave more than 90% weevil survival as compared to cypermethrin and fipronil which are commonly used insecticides for controlling bunch moth *T. mundella* in the oil palm plantation. They can be recommended as alternative insecticides to be used in oil palm plantation because of very little hazardous effect on the oil palm pollinator weevil, *E. kamerunicus* and the environment. However, high dosage of each insecticide should be avoided as they can reduce the population of non-targeted insects like weevil *E. kamerunicus*.

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