UNIVERSITI PUTRA MALAYSIA

EFFECT OF APPLYING OVERDOSE Bacillus thuringiensis (BACILLALES: BACILLACEAE) BASED PESTICIDE ON SURVIVAL RATE OF OIL PALM POLLINATOR WEEVIL, Elaedobius kamerunicus (COLEOPTERA: CURCULIONOIDEA)

WONG CHUNG HUNG

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By

WONG CHUNG HUNG

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

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DEDICATION

This study is wholeheartedly dedicated to the Almighty God, thank you for the guidance, strength, power of mind, protection and skills and for giving us a healthy life. All of these, I offer to you.

Next, I dedicated this book to our beloved parents, who have been my source of inspiration and gave us strength when I thought of giving up, who continually provide their moral, spiritual, emotional, and financial support.

And lastly, to my brothers, sisters, relatives, mentor, friends and classmates who shared their words of advice and encouragement to finish this study.

ABSTRACT

The natural pollinator of oil palm, *Elaeidobius kamerunicus* have been introduced into oil palm plantation in Malaysia to increase the oil palm yield. The application of biopesticide (*Bacillus thuringiensis,* Bt) to control the pest population is often regarded as harmless to a beneficial insect like pollinating weevil. However, it is not known if increased biopesticide dosage will negatively affect weevil. Therefore, this study was carried out to determine the survival rate of weevil when excess dosage of *B. thuringiensis* pesticide was applied. The result of this study found that increasing of *B. thuringiensis* biopesticide concentration on artificial diet from 94.27 ppm to 104715 ppm in dosage did not have adverse effect on *E. kamerunicus* survival. However, the survival rate of *E. kamerunicus* had significant dropped when *B. thuringiensis* biopesticide applied on the weevils' natural diet, oil palm male inflorescence. This indicates overdose application of *B. thuringiensis* biopesticide might have adverse effect on weevil population in the long term. Thus, pest management in oil palm plantation needs to review the effect of *B. thuringiensis* pesticide on pollinating weevil in order to sustain the oil palm industry.

ABSTRAK

Pendebunga semulajadi kelapa sawit iaitu *Elaeidobius kamerunicus* telah diperkenalkan ke ladang kelapa sawit di Malaysia untuk meningkatkan hasil kelapa sawit. Penggunaan bio-racun serangga (*Bacillus thuringiensis*, Bt) untuk mengawal populasi makhluk perosak sering dianggap sebagai tidak berbahaya kepada serangga yang bermanfaat seperti pendebunga kumbang. Walau bagaimanapun, kesan penggunaan racun serangga *B. thuringiensis* dalam dos yang tinggi terhadap kumbang pendebunga masih belum diketahui. Oleh itu, kajian ini dijalankan untuk menentukan kadar kematian kumbang jika racun serangga *B. thuringiensis* digunakan dalam dos yang lebih tinggi daripada dos yang disyorkan. Hasil kajian ini mendapati bahawa peningkatan dos bio-racun serangga *B. thuringiensis* dalam makanan buatan dari 94.27 ppm sehingga 104715 ppm tidak memberi kesan buruk terhadap kebertahanan hidup *E. kamerunicus*. Walau bagaimanapun, kadar kebertahanan hidup pendebunga kumbang menurun secara ketara apabila bio-racun serangga *B. thuringiensis* disebur ke atas makanan semulajadi kumbang iaitu bunga jantan kelapa sawit. Ini menunjukkan bahawa penggunaan *B. thuringiensis* racun serangga berlebihan mungkin memberi kesan buruk kepada populasi kumbang pada jangka masa panjang. Oleh itu, pengurusan serangga perosak di ladang kelapa sawit perlu mengkaji semula kesan penggunaan berlebihan bio-racun serangga *B. thuringiensis* untuk menjaga kelestarian industry kelapa sawit.

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APPROVAL SHEET

I certify that this research project report entitled "Effect of Applying Overdose *Bacillus thuringiensis* (Bacillales: Bacillaceae) Based Pesticide on Survival Rate of Oil Palm Pollinator Weevil, *Elaedobius kamerunicus* (Coleoptera: Curculionoidea)" has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Science, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATION

ANOVA	Analysis of variance
SAS	Analytics software & solutions

CHAPTER 1

INTRODUCTION

Elaeidobius kamerunicus or weevil (Coleoptera: Curculionoidea) is an introduced oil palm pollinator that improved pollination and increased fruit set and yield by 20% in Peninsular Malaysia and 53% in Sabah (Teo 2015). The introduction of *E. kamerunicus* into Malaysia in 1980 had produced several years of high yield of oil palm. However, it was noted that since late 1980 there was a recurrence of which poor fruit set and abortive bunches (Teo 2015). The question has risen over whether the cause factors are climatic, and rainfall related or due to the adverse effect of the pesticide used that was thought to be weevil friendly.

The misuse of pesticide in oil palm plantation that do not follow the active period of pest insects may affect the beneficial insect as they may have different active period. Prasetyo *et al.* (2017) stated that pesticide applications during daytime, the weevil will directly be exposed to the toxins in the pesticide since the weevils are active during the day in the anthesising male and female inflorescence of oil palm. Therefore, applying the pesticide during the active period of beneficial insect will cause the population of beneficial insect like pollinating weevil to drop. The *B. thuringiensis* biopesticide has been widely used to control the pest in Lepidoptera order. This is because it can produce the crystal proteins called δ-endotoxins that selectively kill the Lepidoptera larvae. An example like Lepidoptera order is oil palm moth, *Tirathaba mundella* that will cause poor fruit set (Prasetyo *et al.* 2017).

Tuo *et al.* (2011) reported that the application of chemical insecticide used to control the population of pest insect will bring negative impact to the population of beneficial insects. The population of weevil decrease in the oil palm plantation also influence the oil palm yield. This situation may cause the yield of oil palm to decrease and the economic loss. Therefore, it is better using biopesticide which is less harmful toward the beneficial insect rather than chemical pesticide that kill all the pest and beneficial insect together. An example is biopesticide like *Bacillus thuringiensis*-based pesticide. The *Bacillus thuringiensis* (Bt) product is regarded as safe for the pollinating weevils, *Elaeidobius kamerunicus* (Ahmad *et al.* 2012). But after several years application *B. thuringiensis* product to control the pest population, the concern of the effect of *B. thuringiensis* biopesticide has risen. The objective of this study was to investigate the effect of overdose application of *B. thuringiensis* based biopesticide on the pollinating weevil.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm Pollinating Weevil, *Elaeidobius kamerunicus*

The oil palm tree bears male and female flowers on separate inflorescences. The pollination of oil palm can be done by wind or insects as pollinators. In early stage of oil palm plantation in Malaysia, the oil palm pollination is depending on wind pollination. The natural insect pollinators example like *Thrips hawaiiensis* and *Pyroderces* sp. but the oil palm yield is low due to their low pollination rate (Wahid and Kamarudin 1997). Hand pollination was established in order to raise the oil palm yield. The oil palm pollen was collected, processed and trade in became an abundant sub-industry within the oil palm industry. However, hand pollination is highly labour cost (Syed *et al*. 1982). The adoption of the weevil, *Elaeidobius kamerunicus* in oil palm industry is to solve poor natural pollination problem in Malaysia (Teo 2015).

This natural pollinator, *E. kamerunicus* is from the Africa (Susanto *et al*. 2014). After a few research studies, *E. kamerunicus* was found to be host-specific to oil palm (Saravana *et al*. 2016). Then, *E. kamerunicus* was brought into Malaysia under the quarantine care of the Department of Agriculture in 1980 (Kang and Zam 1982). After verification of the host-specific process of *E. kamerunicus*, there are two states in Malaysia that is Johor and Sabah were first released the pollinating weevil into oil palm estates in February and March 1981. By April 1982, *E. kamerunicus* had exist in almost all the oil palm estates in Malaysia. Syed (1977) conduct a study in Cameroon, Central Africa, he found that weevil, *E. kamerunicus* was the main pollinator for oil palm. It was the most abundant species in both wet and dry seasons and it's had highest capacity of pollen cartage of all pollinators (Syed 1979).

There are a few positive effects after introduce *E. kamerunicus* in oil palm plantation (Basri 1984):

i. Assisted pollination not necessary.

ii. Significant development in fruit set from an average of 52% to 71%.

iii. Raise in fruit to bunch ration from an average of 57.7 to 64.7%.

iv. Raise in mean bunch weight from 14.6 to 18.7kg.

v. Enhancement in oil to bunch ration from a mean of 23.3 to 25.4%

vi. Enhancement in kernel to bunch ratio from 4.6 to 6.6%

Elaeidobius kamerunicus is totally rely on the oil palm male inflorescence to survive. Adult weevils ingest oil palm pollen and lay eggs in anthesizing male inflorescences. The weevil larvae keep and eat up the decomposing male inflorescence and pupate within the spikelets of the flowers, emerging as adults about 10 days later (Tuo *et al.* 2011).

The term for quantifying the weevil population is called pollinator force that is measure of the total number of adults per unit area. The minimum level of population need for adequate pollination is called critical pollinator force (Wahid and Kamarudin 1997). There are few assumptions on the relationship between local pollinators to the *E. kamerunicus* in the oil palm plantation although it has not been studied specifically in the present time. The natural oil palm pollinator, *E. kamerunicus* compete same food source that is male inflorescence with the other two local pollinators that are *T. hawaiiensis* and *Pyroderces*. sp. This situation causes the interspecific competition to happen between these three species and it has probability of the displacement of the local pollinators by *E. kamerunicus*. But for these three competing species above maybe co-exist based on their different species characteristic (Wahid and Kamerudin 1997). They may have different demand of habitat for pupation; *T. hawaiiensis* pupates in the soil while both *E. kamerunicus* and *Pyroderces*. sp pupates in post-anthesis male spikelet. In weather aspect, the *T. hawaiiensis* can increase its population quickly during dry season than *E. kamerunicus* as they grow well during rainfall season. During dry season, the *T. hawaiiensis* help to do the pollination activity as the pollination activity of weevils is decreased. Besides, the different of time of activity between *E. kamerunicus*, *T. hawaiiensis* and *Pyroderces*. sp also help them to be co-existence. The *E. kamerunicus* and *T. hawaiiensis* active during daytime while *Pyroderces*. sp only active for two to three hours after half an hour's sunset (Wahid and Kamerudin 1997).

Saravana *et al*. (2016) report that oil palm is a monoecious plant as it has male and female inflorescences. The oil palm pollination can be depending on wind and insects. There are wide range of insect's species act as natural pollinating agents, one of the most predominant species is *E. kamerunicus*, an oil palm pollinating weevil. The introduce pollinating weevil had improved fruit set from 36.9 to 56.0 % leading in 40% raise in fruit bunch weight. The characteristics of pollinating weevil during pollination need to be study so that it can help to develop the plant protection system and sustain the pollinating weevil for a better performance in pollinating.

Oil palm male inflorescence starts with the opening of florets at the base of male inflorescence during the stage of pollen production of anthesis flower. The florets on the spikelet will slowly opening, it takes three days for all the florets on the spikelets to open. The weevil will start to visit or stay on the oil palm male inflorescence after the florets of the male inflorescence begin to open. The weevil number raise quickly on the second and third day of anthesis and then reduced from the fourth day to sixth day (Saravana *et al*. 2016). Yue *et al*. (2015) stated that the *E. kamerunicus* more abundance found on oil palm male inflorescence than female inflorescence. The adult weevil prefers to live on the oil palm male spikelets for mating and oviposition at dusk (Yue *et al*. 2015).

The anthesis oil palm male inflorescence release a strong aniseed odour to attract the pollinating weevil. This situation can make the pollination of oil palm success. The receptivity of the flower normally keeps on for about 2 to 3 days. The adult weevils are attracted to female flowers by their odour, this is similar to male inflorescences, but they only stay on the female inflorescence for a short duration of time (Saravana *et al*. 2016).

The weevils can carry pollen that sticks all over its body and transfer them to the stigma of the oil palm female inflorescence when they are receptive. However, they do not cause any injury to the female inflorescence. The weevil pollination activities also pollinated the inner flowers in the spikelet. Therefore, the oil palm can produce fully fertilized fruit lets with well-developed kernel and embryo (Saravana *et al*. 2016).

Poor pollination occurs in the young and isolated plantations due to low quantity of oil palm male inflorescence. The oil palm male inflorescence is a habitat for weevils to feeding and breeding. The wind pollination on oil palm is difficult for the low canopy of young plantation acts as a barrier to free air movement. This situation also causes the fruit bunches not well developed because the fruit are either partheniocarpic the smaller fruits produce without kernel or the fruit stopped to grow or decaying. The pollination in aged oil palm plantation do not have problem because the sex ratio declines from 90% of young plantation to about 50% and the taller oil palm tree promote wind pollination (Saravana *et al*. 2016).

There have a lot of reports about the reduction of oil palm yield since the late 1980s after introduction of *E. kamerunicus* in oil palm plantation that have given a high yield of fruit set. The oil palm industry in Pamol, Sabah had losses of more than RM300,000 in 1993 because to poor fruit set. They also found that poor fruit set related to seasonal change, the weevil population and weevil pollination activity may be influence by the wetter weather. Sabah also has been reported facing these problems on the field in 1998 (Tuo 2015). Sarawak and Peninsular Malaysia also reported that the poor fruit set and abortive bunches of male inflorescences because poor pollination of weevil. Based on this situation, there may has other factors that influence the poor pollination problem beside of rainfall and climatic pattern (Teo 2015).

The other factors that cause the poor fruit set is inadequate production of good pollen by oil palm. Another is decreased pollination activity by the pollinating weevil, or a combination of both. There was not proof for the decrease in pollen viability in the oil palm field. The imbalance of sex ratio skew of the oil palm that the female inflorescences is more than male inflorescences will cause low pollen quantity. Oil palms produce male and female inflorescences in alternating cycles, but using modern planting techniques and the suitable condition, the female inflorescence cycle is increased for yields by reduce the time of the male inflorescence cycle. The lower male inflorescence density per hectare will reduce the population of the weevil as the weevil is so reliant on the male inflorescences for breeding sites (Teo 2015).

Predation and parasitism are also needed to consider as the also affect the *E. kamerunicus* populations. Rats prey on weevil larvae by feeding on the post-anthesised male inflorescences. The lifespan of *E. kamerunicus* was cut back due to the infection of internal parasitic nematode, *Elaeolenchus parthenonema*. It has also been suggested that the low resistance of the weevil to parasitism is due to low levels of genetic diversity within the founder population (Teo 2015).

2.2 Current management and research

Manually assisted pollination is required in some areas wherever poor fruit set has been systematically problematic. This practice is believed to be a short-term result to the problem, as assisted pollination is labour intensive. It is hoped that assisted pollination can "break" the reproduction cycle of the palms and stimulate the male inflorescence phase after adequate pollination throughout the female inflorescence phase (Teo 2015).

There are several strategies that are currently being researched. Reducing planting of palms with high feminine traits and desegregation with palms that are powerful male is also some way to handle the problem. Planting materials may also be selected from a more diverse genetic background to decrease the chances of high synchronised inflorescence cycle behaviour between palms so that enough male inflorescences are available all year round (Teo 2015).

Fatihah *et al.* (2018) had stated that the number of male inflorescence and spikelet plays an important part in determination of the population abundance of *E.kamerunicus* per ha. Although the *E. kamerunicus* is more likely active in wet season, but the impact of high rainfall toward *E. kamerunicus* population still not yet recorded.

For a longer-term resolution, we must also enhance our knowledge of oil palm pollinators. In-depth studies are needed and are underway on the weevils to further understand the current problems faced by *E. kamerunicus*. With that, we hope that a solution can be found to double up weevil numbers and if required, to seek out alternative pollinator to *E. kamerunicus* (Teo 2015).

2.3 Life cycle of *Elaeidobius kamerunicus*

Elaeidobius kamerunicus (Faust) is a tiny weevil belonging to the order Coleoptera of the family Curculionidae. The life cycle of the weevil is completely related to the male inflorescences of the oil palm. The weevils laid the eggs in the feeding pit on the corolla of flowers. The eggs of weevil are whitish, oval with a smooth, delicate chorion. Normally one flower can find in egg but frequently two eggs are also seen. Each female weevil lays average 35 eggs during its lifetime. The egg hatches within one or two days and the first larval instar feeds on the inner tissue of filament of male flowers. The larva of weevil has three larval instars. The larva with second instar moves to the base of the flower, where feeding on the soft tissue is resumed (Saravana *et al*. 2016).

The egg and the first two larval stages are completed in three days. The larva with third instar larva feed voraciously and consume from the base of anther tube upwards. In this way the third instar larva of weevil may eat up 5 to 6 flowers in 5 to 8 days of life. The pupation of weevil lasts for 3 days in the consumed flower. Thus, the egg to adult weevil can completed within a period of 11-14 days, but sometimes as long as 20 days. They feed and breed only on male inflorescences after the flowers have released pollen and begin decomposing. The adults chew another filaments or anther tubes of opened flowers. This insect is host specific and develop only on oil palm (Saravana *et al.* 2016).

In India, there is population fluctuation of weevil occurs during summer month (April, May, June) although the weevils are spread well in most of the oil palm plantations. The wet season (July, August, September) is more favourable for the population increase. However, they start building up as soon as weather conditions are conducive (Saravana *et al*. 2016).

There are some natural predators that prey the weevils and influence the population of weevils. One of the natural predators is earwig, *Chelisoches morio*. It is found shelter on male inflorescences and prey on the immature stages of the weevils. Similarly, the garden lizard, *Calotes versicolor* catch and eat weevils using its sticky tongue. It is usually seen on anthesising male inflorescences on that pollinating weevils congregate in great numbers. During the dry seasons, reduvid bugs, *Sycanus* sp. play a vital role in reducing the number of weevils. In addition, weevils emerging from the spent inflorescences that are attracted and coming towards the anthesising male and female inflorescences are trapped in spider webs and are fed by the different species of spiders.

These situations need a great concern especially during summer months as the weevil population is already less (Saravana *et al*. 2016).

Important management tips of remember for conservation suggested (Saravana *et al*. 2016).:

1. The farmers are applying the control measures by using synthetic insecticides like monocrotophs, quinolphos, lamba cyhalothrin, etc, due to increasing insect pest problems. The death of the weevils is detected when these chemicals are sprayed unsystematically. In order to conserve the population of weevil, the criteria for timing and mode of application of insecticides in the oil palm gardens.
    a. Between 9.30 am to 1.00 pm of the period the adult weevils' activities. Therefore, the chemical sprays should be arranging at afternoon hours will help to avoid direct contact of pesticide particles on flying weevils in the farm.
    b. Insecticides like monocrotophos and imidacloprid can be administered through stem injection method instead of direct spraying.
    c. When using the sprayer nozzle should prevent directing the towards the crown region of the palm, especially on male and female inflorescences while spraying as it causes severe mortality of weevils.
2. The adult weevils usually come out from the spent male inflorescences around 11 to 20 days after the first day of anthesis. Hence, it should be careful when cleaning the crown region to avoid the removing the inflorescences which are of less than 20 days old (after the first day of anthesis).

3. The spider webs are existing around the crown region of the inflorescences will trap the weevils that just emerged from the spent inflorescences; Therefore, care must be taken in conserving the weevils and to promote free activity.
4. The newly planted oil palm plantation after 3 years needs to distribute the pollinating weevils. The collection of spent spikelets that have larva and pupa of weevils from the older oil palm plantation and release to the new plantation area. This is common method that introduce the weevils to new oil palm plantation site (Saravana *et al*. 2016).

Tuo *et al*. (2011) stated that the *E. kamerunicus* has 7 stage in lifecycle that is egg stage, nenonate larvae (Lo), larva stage 1 (L1), larva stage 2 (L2), larva stage 3, nymph and adult stage. The *E. kamerunicus* will mature in sexual at 95.5 ± 0.27 hours or 4 days after pupation. The total day for sexual maturity after adding larval stage duration is 14.27 ± 0.35 days. The average life *E. kamerunicus* is 59.18 ± 8.53 days. The female and male adult weevil life span is 31.22 ± 6.47 days and 27.96 ± 2.99 respectively.

Table 2.1 The duration of the developmental stages at *E. kamerunicus* and *E. Plagitus*. (Tuo *et al.* 2011).

Stages	*E. Kamerunicus*	*E. Plagiatus*
Egg – L0 (hours)	5.84±0.06	5.39±0.09
L0 – L1 (hours)	18.16±0.06	12.07±0.13
L1 – L2 (day)	1.24 ± 0.12	0.51 ± 00
L2 – L3 (day)	1.08 ± 0.1	2.90 ± 0.04
L3 – N (day)	4.96 ± 0.11	2.63 ± 0.05
N - Ad (day)	2.03 ± 0.037	1.67 ± 0.08
Total cycle duration (day)	10.27 ± 0.34	8.38 ± 0.12

2.4 Toxicity of *Bacillus thuringiensis* to oil palm weevil, *Elaeidobius kamerunicus*

Bacillus thuringiensis (Bt) is a natural, soil-borne bacteria. has been used to control the pest population since the 1950s. It is a gram-positive spore-forming bacterium. It will produce crystalline proteins called delta-endotoxins during its stationary phase of growth. Different species of *Bacillus thuringiensis* produce different toxic protein and cause different insect species dead due to certain protein produced by different species of *Bacillus thuringiensis*. The bacteria will emit the toxic crystal in the insect's gut that is highly alkaline when the bacteria are eaten by certain insects. The toxic crystals will be blocking the system that protect the pest's stomach from its own digestive juices. The pest's stomach is penetrated due to the crystal protein and the pest dies (Schunemann *et al.* 2014).

Bacillus thuringiensis (Bt) proteins have insecticidal properties that can kill the insect. It has been packaged into biopesticide product and used commercially for more than 40 years. The global market is 90% majorities make up by Bt-based biopesticide. (Ahmad *et al.* 2012). The Bt-based biopesticides are commonly applied and are known as replacement insecticides to chemical insecticides. This is because Bt-based biopesticide is safety to non-target organism and it is still work toward the pest species developed resistance to chemical insecticides (Ahmad *et al.* 2009).

Bt-based biopesticide normally used to control the pest insect for Lepidoptera order as the crystal protein of *B. thuringiensis* is very toxic to them. But it is non-dangerous to other non-target insect or beneficial insect, animals and even human. However, the safeness of Bt-based biopesticide need to study from time to time because the characteristic of *B. thuringiensis* towards non-target organism may change. The future study needs to carry out to more secure that the Bt-based biopesticide when applied in oil palm plantation to control oil palm pests. do not bring any side effect on ecological system and environment (Ahmad *et al.* 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 Weevil, *Elaeidobius kamerunicus*

Elaeidobius kamerunicus specimens were collected in the oil palm plantation site in Sebauh, Bintulu, Sarawak. The oil palm male inflorescence was collected as the *E. kamerunicus* live on it. Adult *E. kamerunicus* were collected for this study as the adult stage of *E. kamerunicus* is the longest stage among other stages of *E. kamerunicus*. During the anthesis of oil palm male inflorescence, the weevil activity is mostly founded during inactive period (07:00 - 08:00), substantial activity (11:00 – 12:00), and peak activity (17:30 – 18:00). (Wahid and Kamarudin 1997; Yue *et al*. 2015). Therefore, the pollinating weevil is the pollinating weevil was collected at daytime as they were more active at daytime.

3.2 Bioassay of *Bacillus thuringiensis* biopesticide on *Elaeidobius kamerunicus*, weevils.

In this study, the bioassay was carried in the laboratory and two types of media were used in bioassay. Media 1 was an artificial diet for *Lepidoptera* order, *Tirathaba mundella* (oil palm moth) while media 2 was an oil palm male inflorescence. There were two bioassays had carried out by using media 1 while only one bioassay carried out by using media 2. Artificial diet was prepared according to the ingredient listed in Table 3.1. The reason for artificial diet for *Lepidoptera* was used because the artificial diet main ingredient is oil palm male inflorescence as the natural food sources of pollinating weevil was oil palm male inflorescence. The pollinating weevil also can consume the artificial diet. The advantages of using artificial diet to do the bioassay is it can produce in laboratory, and the sterilization of the artificial diet can be done by autoclave to reduce the contamination of bacteria or fungus, and the artificial diet can be easily replaced by new artificial diet.

For media 2 (oil palm male inflorescence), the reason using oil palm male inflorescence as media is to obtain more accurate reading as the pollinating weevil is live in oil palm plantation and they are consumed and live on the oil palm male inflorescence. Ahmad *et al*. 2009 had stated collect the oil palm male inflorescence as food media for the weevil to do the bioassay of effect of *B. thuringiensis* biopesticide on the pollinating weevils. The pollinating weevils were more likely to consume on oil palm male inflorescence compare to artificial diet. The oil palm male inflorescence was collected from the oil palm estate during the process of collecting the pollinating weevils. The oil palm male inflorescence was collected from oil palm estate and dry in oven (37˚C) overnight to prevent the microorganism growth by reduce the moisture content of oil palm male inflorescence. The limitation using the oil palm male inflorescence was difficult to get fresh oil palm male inflorescence as the sampling process to continuously carried out to get the fresh and anthesis oil palm male inflorescence. In addition, the oil palm male inflorescence started to grow mold or fungus after a few days harvest from the oil palm tree.

3.2.1 Preparation of Artificial Diet

For media 1 (artificial diet), all the quantity needed to prepare the diet was weighed. The diet was prepared in 1L beaker and cooked at hot plate. The ingredients were added based on the order and cooked until it boils. Then using aluminium foil to cover on the mouth of beaker. Then the diet was autoclaved at the temperature of 121˚C. This process is to ensure the diet was in sterilize and to prohibit the microorganism growth on it. The ascorbic acid and Vanderzant vitamin were degrade or destroy if they added into the artificial diet before autoclave. Therefore, the syringe filter was used to sterile the ascorbic acid and Vanderzant vitamin before were added into artificial diet. The diet was mixed well and then 50 mL artificial diet was measured and then pour into the plastic container (7 cm X 2.8 cm, diameter X height).

Table 3.1 Composition of artificial diet for the rearing of *E. kamerunicus* (Calvin 2017).

Diet	Ingredient	Quantity
I	Casein	126 g
	Sucrose	135 g
	Wheat germ/ Palm kernel cake	175 g
II	Potassium sorbate	36 g
	Methylparaben	4 g
	Cellulose	25 g
	Wesson's salt	5.4 g
III	Aureomycin	36 g
	Ascorbic acid	4 g
	Crude palm oil	14 g
	Vanderzant vitamin	26 g
	45% KOH solution	9 mL
	40% Formalin	3 mL
IV	Agar	96 g
	Distilled water	Up to 3000 mL

3.2.2 Preparation of *Bacillus thuringiensis* biopesticide

The *Bacillus thuringiensis,* Bt biopesticide were preared in six types of concentrations that were C1 (45 µL/50 mL or 94.24 ppm), C2 (75 µL/50 mL or 157.07 ppm), C3 (105 µL/50 mL or 219.90 ppm), C4 (135 µL/50 mL or 282.73 ppm), C5 (165 µL/50 mL or 345.56 ppm), C6 (1 mL/50 mL or 104715 ppm). C1 is under the recommended dosage. C2 is the recommended dosage and C3 to C6 are overdose concentration. The value in ppm is stand for the amount to crystal protein of Bacillus thuringiensis in the treatment. Both bioassay for media 1 and media 2 was using same *B. thuringiensis* concentration as treatment. The pipette was used to get exact volume of *B. thuringiensis* biopesticide and added into the artificial diet (media 1) with the volume of 50mL and mixed well before it solidified. After the diet was cooled down, the diet was store at the 4°C at refrigerator to prevent the microbial growth. The negative control of artificial diet was not added any concentration of *B. thuringiensis* biopesticide.

For media 2 (oil palm male inflorescence), *B. thuringiensis* biopesticide were using pipette to get the exact volume and then mixed in the 50mL distilled water. Next, each concentration of *B. thuringiensis* biopesticide solution were poured into small sprayer that already labelled. The negative control for this bioassay was using the 50mL distilled water without adding any concentration of *B. thuringiensis* biopesticide and pour into the small sprayer. Then, the small sprayer was sprayed on the oil palm male inflorescence and dry in room condition for 30 minutes. After that, the oil pam male inflorescence was directly put into the plastic container (4.5 cm X 17 cm X 12 cm, height X long X width) that had pollinating weevil.

3.2.3 Weevils Rearing and Observation

There were seven treatments carried out for bioassay using media 1 and media 2. Seven treatments were negative control, C1, C2, C3, C4, C5, and C6 done in this bioassay. Each treatment had 3 replications and each treatment had 10 pollinating weevils. The rearing condition for the three bioassays was controlled at the room temperature of 25 to 30° C and 12 hours light: 12 hours dark photoperiod in a relative humidity (RH) of 67 to 90%. The artificial diet was replaced after 3-4 days. The behaviour of weevils on the artificial diet and oil palm male inflorescence was observed and the number of survival of weevils was counted every day then tabulated in the table.

In this study, the cut off line for the three bioassays for statistical analyses was chosen that was Day 3 after the treatment. This is because the pollinating weevil may dead due to other factors. Example like the life cycle of the pollinating weevil had finish, having stress during sampling process or the weevil cannot adapt to new environment.

The experiment design for this study was completely randomised design (CRD). All the data of survival rate of weevil against the *B. thuringiensis* biopesticide was using SAS version 9.3 to do statistical analyses. The data for survival rate after expose to *B. thuringiensis* biopesticide was analyzed by one-way analysis of variance (ANOVA), mean of the parameters were compared by Duncan's Multiple Range Test ($p < 0.01$). Independent-sample t test was used to calculate mean and standard error for each parameter (Ahmad *et al*. 2009).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Bioassay of *Bacillus thuringiensis* based biopesticide against the survival rate of weevil.

In this study, bioassays of *Bacillus thuringiensis,* Bt based biopesticide against the survival rate of weevil with 3 replications were carried out. Two bioassays were carried on of artificial diet and one bioassay on oil palm inflorescence. The percentage of survival rate of weevil effect of *B. thuringiensis* biopesticide on the survival of *Elaeidobius kamerunicus* survival rate shown in Table 4.1.

Table 4.1 Effect of *B. thuringiensis* biopesticide against survival rate of pollinating weevil on Day 3.

Pesticide concentration (ppm)	**Survival rate of pollinating weevil, %**		
	Bioassay 1 (Artificial Diet)	**Bioassay 2 (Artificial Diet)**	**Bioassay 3 (Oil Palm Male Inflorescence)**
Negative Control (0)	$66.67^{a} \pm 3.33$	$93.33^{a} \pm 6.67$	$80.00^{a} \pm 0.00$
C1 (94.24)	$80.00^{a} \pm 10.00$	$86.67^{a} \pm 6.67$	$16.67^{b} \pm 8.82$
C2 (157.07)	$76.67^{a} \pm 3.33$	$86.67^{a} \pm 3.33$	$26.67^{b} \pm 14.53$
C3 (219.90)	$73.33^{a} \pm 8.82$	$93.33^{a} \pm 3.33$	$30.00^{b} \pm 5.77$
C4 (282.73)	$76.67^{a} \pm 8.82$	$93.33^{a} \pm 6.67$	$46.67^{ab} \pm 8.82$
C5 (345.56)	$80.00^{a} \pm 5.77$	$96.67^{a} \pm 3.33$	$26.67^{b} \pm 8.82$
C6 (104715)	$53.33^{a} \pm 3.33$	$80.00^{a} \pm 5.77$	$36.67^{ab} \pm 13.33$

Notes: Values for percentage of survival rate are means ± SE. The values followed by the same letter do not differ significantly (p = 0.01, Duncan's Multiple Range Test).

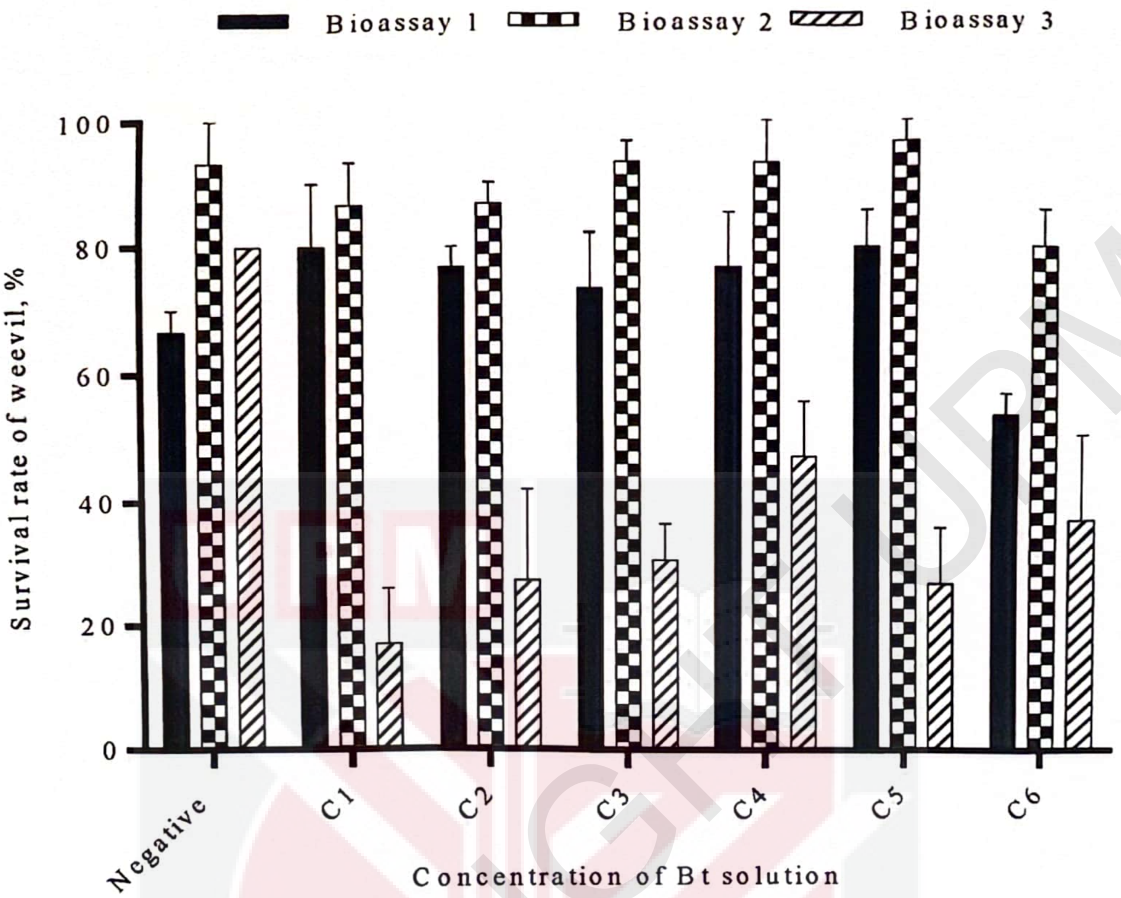

Figure 4.1 Effect of *B. thuringiensis* biopesticide against survival rate of pollinating weevil on Day 3.

Based on artificial diet bioassays (Bioassay 1 and 2), the survival rate for weevils across the six concentration of *Bacillus thuringiensis,* Bt biopesticide range from 94.24 ppm to 104715 ppm were not significant different from negative control based on p = 0.01 on the 99% confidence level.

For the bioassay 3 using oil palm male inflorescence as media, the weevil survival rate for *B. thuringiensis* biopesticide concentration range from 94.24 ppm to 104715 ppm were significantly lower than negative control at confidence level 99% (α = 0.01). The result showed that *B. thuringiensis* biopesticide may have negative effect on the survival rate of pollinating weevil.

From this study, the bioassay with artificial diet and bioassay with oil palm male inflorescence showed different result on the *Bacillus thuringiensis*, Bt biopesticide against the survival rate of pollinating weevil, *Elaeidobius kamerunicus*. The bioassay with artificial diet (First Bioassay and Second Bioassay) showed there were not significant effect ($p = 0.01$) of *B. thuringiensis*, Bt biopesticide on the survival rate of pollinating weevil. While the bioassay with oil palm male inflorescence showed there was significant effect ($p = 0.01$) on the survival rate of pollinating weevil. This is probably due to nature of diet provided in the bioassay.

Oil palm male inflorescence is natural diet to weevil therefore it was more likely the weevils to feed on it. When the pollinating weevil fed more on the oil palm male inflorescence, it also caused them to greater exposure to *B. thuringiensis* biopesticide than the weevil fed on artificial diet. Higher exposure to *B. thuringiensis* caused more side effects on weevil (refer to Figure B.1) and higher mortality.

The result of this study agrees with the hypothesis suggested by Teo (2015). He stated that the oil palm yield had reduced after a few years' introduction of *Elaeidobius kamerunicus* to the oil palm plantation. This situation happen may due to poor pollination of weevil. The population of weevil may be dropped due to climate pattern and rainfall (Teo 2015). The application of chemical insecticide to control the pest population may also reduce the population of beneficial insect like pollinating weevil (Tuo *et al.* 2011).

In other study, Yusdayati and Hamid (2015) stated that the directly apply *B. thuringiensis* biopesticide to weevil shown harmful to their population, however *B.*

thuringiensis biopesticide do not affect the weevil's egg. Weevil can still recover their population in the oil palm plantation if period to application *B. thuringiensis* have been scheduled (Yusdayati and Hamid 2015).

The result of this study shown that regardless the dosage of biopesticide use, the survival rate of pollinating weevil, *E. kamerunicus* will be negatively affected when they fed on the treated male inflorescence. However, there was a limitation to this study. The number of bioassay replications was not sufficient due to difficulty to obtain weevil. As weevils are valuable asset to oil palm industry, it is not easy to get permission to catch large number of weevils for bioassay. However, the result showed strong indication of negative effect of *B. thuringiensis,* Bt pesticides on weevils.

CHAPTER 5

CONCLUSION

This study showed that when *Bacillus thuringiensis*-based pesticide applied on artificial diet did not cause negative effect on the population of pollinating weevil, *Elaeidobius kamerunicus*. However, when the *B. thuringiensis*-based pesticide was applied on oil palm male inflorescence, which is the natural diet to weevil, the survival rate significantly lower than the negative control. It strongly indicates that *B. thuringiensis* (Bt) may cause adverse effect on the pollinating weevil. Therefore, the application of *B. thuringiensis* should be carefully applied and scheduled based on the knowledge of active period of the pollinating weevil to avoid *B. thuringiensis* (Bt) exposure to the weevils. Low population of pollinating weevil will cause poor pollination on oil palm. In order to sustain the oil palm industry, further study needs to be carried out to prove this claim and strategize an application schedule.

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APPENDICES

Table 1 Raw data of Bioassay 1 from Day 1 to Day 3.

Pesticide concentration (ppm)	Survival rate of pollinating weevil, %			
	Replication	Day 1	Day 2	Day 3
Negative control (0)	R1	80	80	70
	R2	90	70	60
	R3	80	80	70
C1 (94.24)	R1	100	90	90
	R2	90	90	90
	R3	80	80	60
C2 (157.07)	R1	90	90	70
	R2	100	90	80
	R3	90	80	80
C3 (219.90)	R1	100	90	90
	R2	80	70	60
	R3	70	60	70
C4 (282.73)	R1	100	80	90
	R2	80	80	80
	R3	80	60	60
C5 (345.56)	R1	90	90	80
	R2	90	90	90
	R3	80	80	70
C6 (104715)	R1	100	70	50
	R2	80	60	50
	R3	80	60	60

Table 2 Raw data of Bioassay 2 from Day 1 to Day 3.

Pesticide concentration (ppm)	**Survival rate of pollinating weevil, %**			
	Replication	**Day 1**	**Day 2**	**Day 3**
Negative control (0)	R1	100	100	100
	R2	100	100	100
	R3	100	100	80
C1 (94.24)	R1	100	80	80
	R2	90	80	80
	R3	100	100	100
C2 (157.07)	R1	90	90	80
	R2	100	100	90
	R3	100	100	90
C3 (219.90)	R1	90	90	90
	R2	100	100	100
	R3	90	90	90
C4 (282.73)	R1	100	100	80
	R2	100	100	100
	R3	100	100	100
C5 (345.56)	R1	100	100	100
	R2	100	100	100
	R3	100	100	90
C6 (104715)	R1	80	80	80
	R2	80	80	70
	R3	100	100	90

Table 3 Raw data of Bioassay 3 Day 1 to Day 3.

Pesticide concentration (ppm)	**Survival rate of pollinating weevil, %**			
	Replication	**Day 1**	**Day 2**	**Day 3**
Negative control (0)	R1	80	80	80
	R2	80	80	80
	R3	80	80	80
C1 (94.24)	R1	100	50	20
	R2	50	50	0
	R3	60	60	30
C2 (157.07)	R1	80	60	30
	R2	80	80	50
	R3	60	60	0
C3 (219.90)	R1	90	90	30
	R2	50	30	20
	R3	90	80	40
C4 (282.73)	R1	80	70	50
	R2	90	70	60
	R3	70	70	30
C5 (345.56)	R1	30	30	30
	R2	50	50	10
	R3	90	90	40
C6 (104715)	R1	100	70	10
	R2	100	100	50
	R3	100	100	50

Plate 1 Image of weevil condition after the treatment of *Bacillus thuringiensis* biopesticide.

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Effect of Applying Overdose *Bacillus thuringiensis* (Bacillales: Bacillaceae) Based Pesticide on Survival Rate of Oil Palm Pollinator Weevil, *Elaedobius kamerunicus* (Coleoptera: Curculionoidea)" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture, Food Science, Universiti Putra Malaysia Campus Bintulu Sarawak and published in the form approved by the faculty.

WONG CHUNG HUNG

Date: