POPULATION DYNAMICS OF TROPICAL BED BUG (CIMEX HEMIPTERUS F.) (HEMIPTERA: CIMICIDAE) FED ON DIFFERENT HUMAN BLOOD TYPES

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

POPULASI DINAMIK PEPIJAT TROPIKAL (CIMEX HEMIPTERUS F.) (HEMIPTERA: CIMICIDAE) YANG DIBERI MAKAN JENIS DARAH MANUSIA YANG BERBEZA

ABSTRAK

Pepijat adalah sejenis ektoparasit yang hanya memakan darah semata-mata. Kesemua tahap tidak matang haiwan ini memerlukan darah untuk proses pertukaran kulit dan pepijat dewasa memerlukannya untuk pembiakan. Sistem pemakanan buatan dapat memberi makan serangga ini dengan menggunakan darah manusia yang telah tamat tarikh lupus. Kesan tiga jenis komposisi darah; darah penuh (WB), sel darah merah (RBC) dan sel darah merah dicampur dengan plasma (RBP), dikaji untuk menentukan darah yang sesuai bagi sistem pemakanan tiruan, dan dibandingkan dengan kaedah pemakanan secara langsung. Bilangan individu yang hidup dan mati, dan bilangan pepijat dewasa terhasil direkodkan. RBC menyebabkan kematian 72.2% pepijat, diikuti oleh RBP (52%) dan WB (48.7%). Terdapat perbezaan signifikan antara keempat-empat sumber darah bagi bilangan individu yang hidup selepas 7 minggu pepijat diberi makan. Namun, tiada perbezaan signifikan bagi bilangan individu yang hidup antara ketiga-tiga komposisi darah (WB, RBC dan RBP). Jadual hayat telah dibina bagi pepijat yang diberi makan WB melalui sistem pemakanan buatan mengikut jenis darah manusia, A, B, O dan AB. Bilangan telur dan bilangan individu yang hidup direkodkan setiap hari. Analisis statistik bagi peringkat tidak matang mendapati tiada perbezaan signifikan bagi masa yang diambil untuk pepijat menjadi dewasa antara darah A, B, O dan AB. Jadual hayat yang lengkap dibina bagi pepijat yang diberi makan darah A dan AB. Pepijat yang dibiak dengan darah

A dan AB masing-masing mempunyai jangkaan hidup sebanyak 88 dan 105 hari. Kadar penggantian bersih (R_0), purata masa generasi (T), kadar peningkatan serta-merta (r_m), kadar peningkatan terhingga (λ), dan masa penggandaan (DT) bagi pepijat yang dibiak dengan darah A adalah 12.24, 67.84, 0.037, 1.038 dan 18.73. Pepijat yang dibiak dengan darah AB pula menghasilkan R_0 = 12.58, T = 83.36, r_m = 0.030, λ = 1.030 and DT = 23.10. Tiga bahagian badan; lengan, lengan atas dan bahu didedahkan pada gigitan pepijat untuk menentukan kesannya pada kulit dan masa yang diambil untuk kesan tersebut hilang direkodkan. Tiga jenis reaksi kulit iaitu gatal-gatal, bengkak dan lesion direkodkan. Berdasarkan analisis statistic, tiada perbezaan signifikan antara masa yang diambil untuk reaksi kulit hilang bagi ketiga-tiga bahagian badan, begitu juga antara bilangan individu dan jantina. Bagaimanapun, terdapat sedikit perbezaan direkodkan bagi reaksi kulit di mana lesion yang terbentuk di bahu mengambil masa yang lebih lama untuk hilang berbanding lengan dan lengan atas. Rekod juga menunjukkan reaksi kulit yang terbentuk disebabkan oleh gigitan lima ekor pepijat mengambil masa yang lebih lama untuk hilang berbanding seekor dan tiga ekor pepijat.

POPULATION DYNAMICS OF TROPICAL BED BUG

(CIMEX HEMIPTERUS F.) (HEMIPTERA: CIMICIDAE)

FED ON DIFFERENT HUMAN BLOOD TYPES

ABSTRACT

Bed bug is an ectoparasite that feed exclusively on blood. All stages of nymphs require a blood meal before molting, while adults need it for reproduction. Artificial feeding system is an alternative way to feed these insects by using expired human blood supplies from the hospital blood bank. The effects of three types of blood source composition; whole blood, red blood cells and red blood cells mixed with plasma, were investigated to determine the suitable blood source that could be used to feed the bed bugs, with comparison to the direct feeding method. Observations included counts of the number of live and dead individuals, and number of adults produced. Red blood cells caused 72.7% of bed bugs died, followed by red blood cells mixed with plasma (52%) and whole blood (48.7%). There was a significant difference in number of live individuals after bed bug being fed for seven weeks between the four blood sources. However, there are no significant difference between the number of live individuals fed on the three blood compositions (whole blood, red blood cells and red blood cells mixed with plasma). Life table of bed bugs fed through an artificial feeding system according to the human blood type, A, B, AB and O, were constructed. The number of individuals that lived (l_x) were counted and recorded daily as well as the number of eggs laid. Statistical analysis for the development of immature stages showed that no significant difference was observed for the development from egg to emergence of adult when fed on blood type A, B, O and AB.

Age specific life table were constructed for tropical bed bugs reared on blood type A and AB. Bed bugs reared on blood type A and AB had a life expectancy of 88 and 106 days respectively from the egg stage. The net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), finite rate of increase (λ), and doubling time (DT) for bed bugs reared on blood type A were 12.24, 67.84, 0.037, 1.038 and 18.73, respectively. On the other hand, the same parameters calculated for bed bugs reared on blood type AB produced R_0 = 12.58, T = 83.36, r_m = 0.030, λ = 1.030 and DT = 23.10. Three parts of the body; forearm, upper arm, and shoulder were exposed to bed bug bites to determine the type of skin reactions manifestation and the time taken for the skin reactions to disappear. Three types of skin reactions, itch, wheal and lesion, were recorded. There was no significant difference between the time taken for skin reactions to disappear for the three body parts, as well as between the number of individual and gender. However, some differences were observed for skin reactions to disappear where lesions on the shoulder would last longer than forearm and upper arm. Skin reactions from five individuals of bed bugs generated skin reactions that took longer time to disappear compared to one and three individuals of bed bugs.

CHAPTER ONE

INTRODUCTION

Bed bug is an ectoparasite that feeds exclusively on blood of humans, birds and other mammals such as rabbits and cats (Goddard and deShazo 2009, Harlan 2006, Lehane 2005, Montes et al. 2002, Reinhardt and Siva-Jothy 2007). A survey conducted in infested premises in Malaysia and Singapore by How and Lee (2010c) showed that only one species of bed bug was found, the tropical bed bug, *Cimex hemipterus* (Fabricius). Another survey was conducted by Zulaikha et al. (2016) from November 2013 until December 2014 in 11 states of Peninsular Malaysia, also found only one species of bed bug, *C. hemipterus*, infested the targeted premises surveyed in the urban areas where abundant of foreigners were staying.

Meanwhile a survey conducted in some dwelling places in Thailand by Suwannayod et al. (2010) reported that even common bed bug, *Cimex lectularius* (Linnaeus) can be found in tropical area. *Cimex lectularius* (*C. lectularius*) usually found over temperate regions such as the United States, Canada and the United Kingdom (Hwang et al. 2005, Reinhardt and Siva-Jothy 2007). People travelling around may be the key factor that facilitate the distribution of bed bugs around the world (How and Lee 2010a). Most people do not realize that bed bugs have been infested their places until the numbers increase remarkably high. At this point, to control and eradicate this insect will cost more and become more difficult.

Bed bugs are nocturnal creatures that feed and active at night, generally between midnight and 5:00 am depending on the host's schedule and they usually avoid light and hide during the day. However, they will seek host and feed during daytime when hungry (Harlan 2006, Lehane 2005, Miller et al. 2013, Romero et al. 2010, Usinger 1966). After feeding on their host is completed, bed bugs will move to their hiding places or harborage areas which include seams in mattresses, backsides of headboards, and crack and crevices in wall, wooden furniture, floors, even inside electrical sockets and nearby computers (Harlan 2006, How and Lee 2010a, How and Lee 2010c, Hwang et al. 2005, Reinhardt and Siva-Jothy 2007). They spend the majority of their time hiding together in their harborage sites where they will not be seen or disturbed (Harlan 2006, Johnson 1941, Miller et al. 2013, Romero et al. 2010).

Infestation are now become noticeable in the hotel and motel, workplace, transportation vehicles, cinemas and they can be found in any location where people sleep or sit such as airport and bus station (Bernardeschi et al. 2013, Doggett et al. 2012, How and Lee 2010c, Zulaikha et al. 2016). The infestation can be explained by few factors such as exchanging second-hand furniture which is one of the favorite sites for bed bug harborage. Since bed bugs are small, they can be transported by humans in clothing, luggage, and furniture (Delaunay 2012, Reinhardt and Siva-Jothy 2007, Romero et al. 2007, Suwannayod et al. 2010). People might carry along the bed bugs when they travel to places whether for business or pleasure. Insecticide resistance and changes of insecticides used to control bed bugs also contributed to high infestation of this nuisance insect (Davies et al. 2012, Moore and Miller 2006, Suwannayod et al. 2010).

One life cycle of bed bug from first hatch of egg to next offspring (egg) is approximately 6 week under natural conditions. These bugs mate after a blood meal by a process called traumatic insemination where the male pierces the female's abdomen (Harlan 2006, How and Lee 2010b, Reinhardt and Siva-Jothy 2007, Usinger 1966). A single female bed bug can produce 200-500 eggs in her lifetime (Harlan 2006, How and Lee 2010b, Lehane 2005). Adult bed bugs are broadly oval shaped, flat, and can grow to approximately 5–7 mm in length. Adults are brown to reddish brown in color, whereas nymphs are much smaller and light yellow in color. Adult bed bugs have an average life span of 6 months up to 1 year (Goddard and deShazo 2009, Harlan 2006, Reinhardt and Siva-Jothy 2007, Usinger 1966). Bed bugs have five immature stages and every molting requires a blood meal (Barbarin et al. 2013, Harlan 2006, Miller 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966). Previous studies showed that bed bugs can be reared on blood of human, mice, rabbit, guinea pig, bird and chicken (Aak and Rukke 2014, Araujo et al. 2009, Barbarin et al. 2013, Cannet et al. 2015). According to Montes et al. (2002), heparinized blood was found the most suitable for feeding bed bugs using artificial feeding system. After fed on blood and reached engorged weights, the bugs may increase 30% to 50% in length and 150% to 200% in weight (Goddard and deShazo 2009).

Bed bugs affect their hosts' quality of life, causing anxiety, sleeplessness, itchy skin, discomfort, and stress (Barbarin et al. 2013, deShazo et al. 2012, Reinhardt and Siva-Jothy 2007, Suwannayod et al. 2010, Thomas et al. 2004). A person who gets bite by the bed bug usually does not feel the bite because the bugs release biologically active proteins through their saliva (Harlan 2006, Leverkus et al. 2006, Serrão et al. 2008). Bed bug bites must be differentiated from other insect bites such as body lice where bed bug bite

diagnostic clues include clustering and timing (Hwang et al. 2005). Effects of bed bug bites are varying depend on the individual. Symptoms include a raised, inflamed, reddish weal which may itch intensely for several days. The bites can cause small clusters of extremely pruritic, erythematous papules or wheals, vesicle formation, and hemorrhage (Harlan 2006, Reinhardt et al. 2009).

Immediate reactions may appear from 1 to 24 hour after a bite and may last 1–2 days. Delayed reactions usually appear 1–3 days or more after a bite and may last 2–5 day (Harlan 2006, Leverkus et al. 2006). Repeated bites may result in skin lesions and patient may even develop systemic hypersensitivity or even severe hemoglobin loss in some cases. Scratching itchy wounds may cause secondary infections, including folliculitis, cellulitis, or eczematous dermatitis (Harlan 2006, Leverkus et al. 2006, Reinhardt and Siva-Jothy 2007). Reinhardt et al. (2009) reported that some people are insensitive to bed bug bites and that only ~ 80% of the populations are sensitive to bed bug bites. Bed bug could be a vector of a disease theoretically, however they have never been proven to transmit disease to human. For examples, Hepatitis B viral DNA can be detected in this insect up to 6 weeks, but no transmission of this disease infection was found in a chimpanzee model (Hwang et al. 2005). Jupp and Lyons (1987) conducted experiments to assess whether bed bugs and mosquitoes could act as vectors of human immunodeficiency virus (HIV) and they found that these insects are not mechanical vectors of HIV under natural conditions. Goddard and deShazo (2009) reported that no study has showed that bed bug is a vector competence, which mean this insect does not has the ability to acquire, maintain and transmit an infectious agent or virus.

The following are the specific objectives of the three main studies.

Study 1 (Chapter 3): Laboratory rearing of *Cimex hemipterus* F. (Hemiptera: Cimicidae) feeding on different types of human blood compositions (whole blood, red blood cells and red blood cells mixed with plasma) by using modified artificial feeding system.

1. To determine the factors that attract bed bugs to feed on blood meal.
2. To determine the suitable blood source that can be used to feed bed bugs using artificial feeding system.

Study 2 (Chapter 4): Life table analysis of *Cimex hemipterus* F. (Hemiptera: Cimicidae) reared on different types of human blood, A, B, O and AB, by using an artificial feeding system.

1. To determine the development time of each immature stage and total development time of *C. hemipterus* that feed on different types of human blood, A, B, O and AB.
2. To compare adult longevity and fecundity of *C. hemipterus* reared on blood type A and AB using artificial feeding system.

Study 3 (Chapter 5): Skin reactions towards bed bug bites at three parts of human body, forearm, upper arm and shoulder.

1. To determine whether skin reaction appears differently when bed bugs fed on forearm, upper arm and shoulder.
2. To determine the time taken for skin reactions from bed bug bites on forearm, upper arm and shoulder, disappear.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of Bed Bug

The bed bugs belong to family Cimicidae that parasitizes primarily on humans, birds, and bats. Their best-known member is the common bed bug, *Cimex lectularius* (*C. lectularius*) which can be found especially in the temperate region, and *Cimex hemipterus* (*C. hemipterus*), also known as tropical bed bug, normally found in tropical countries as the name suggested (Harlan 2006, How and Lee 2010c, Lehane 2005, Reinhardt and Siva-Jothy 2007, Usinger 1966). Bed bugs are highly specialized species of insect where both female and male, and all of immature stages feed on blood for their survival, growth and reproduction (Benoit et al. 2012, Harlan 2006, Lehane 2005, Miller et al. 2013, Reinhardt and Siva-Jothy 2007). *C. lectularius* and *C. hemipterus* are almost always associated with humans but these species survive well on several different host such as birds, bats, mice, and rabbits. All hosts have a relatively high body temperature and live in enclosed spaces such as room, building or caves (Aak et al. 2014, Barbarin et al. 2013, Harlan 2006, Reinhardt and Siva-Jothy 2007, Suchy and Lewis 2011, Usinger 1966).

Adult bed bugs have a dorso-ventrally flattened body shape, about 6–7 mm long, reddish-brown in color, covered with short hairs, which allows it to crawl and hide in narrow cracks and crevices (Harlan 2006, Miller et al. 2013, Pratt and Stojanovich 1967). The flattened body of bed bugs became engorged once they finished their feeding session which would be as big as twice its original unfed size (Johnson 1941, Reinhardt and Siva-

Jothy 2007). Other prominent external morphological features include the head that bears pairs of 4-segmented antennae, a 3-segmented beak or mouthpart, a broad pronotum, pairs of degenerate wings and three pairs of slender but well-developed legs for rapid movement. The third and fourth segments of the antennae are thinner than either the basal or second segment (Miller 2008, Reinhardt and Siva-Jothy 2007). The pronotum of bed bug is very distinct and usually used for species identification where the pronotum of *C. hemipterus* is not as broad as *C. lectularius* (Khan and Rahman 2012, Pratt and Stojanovich 1967, Usinger 1966). The male adult is differentiated from the female by the asymmetrical pointed tip of the abdomen (Figure 2.1). On the other hand, female bed bug has more rounded tip of the abdomen (Harlan 2006, Miller 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966).

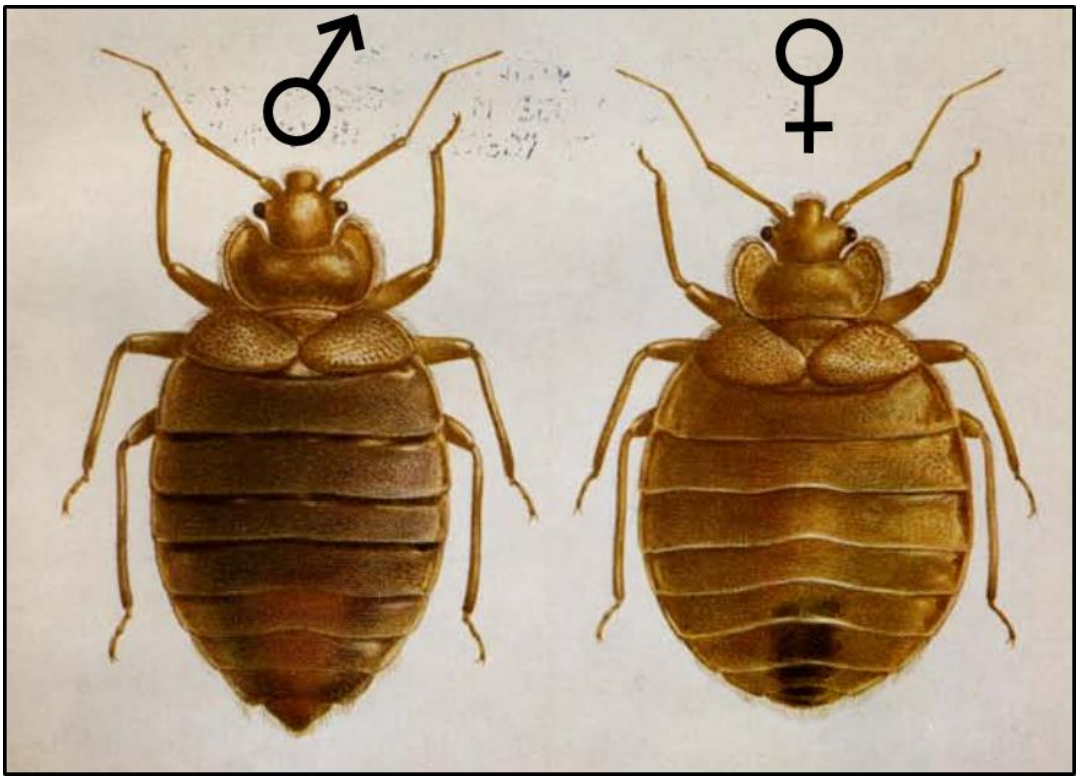

Figure 2.1 Male and female of *C. lectularius* (Usinger 1966).

Bed bugs have a unique way or technique of mating called traumatic insemination, and the females have evolved and developed specific adaptations to it. Traumatic insemination can be defines as copulation process in which the male inseminates through the female abdominal wall, into the body cavity using its needle-like penis and injects a mass of sperms into the mesospermalege, not through the female's genital tract as most of the insects (Lehane 2005, Miller 2008, Reinhardt and Siva-Jothy 2007). The term "traumatic" refers to the integumental wound in the female abdomen after each mating and to the cost of copulation. How and Lee (2010b) reported that unmated males and females lived longer than mated ones, up to four times. The regular female reproductive organ and opening function only in egg laying. Recently fed females attract the males to mate and such female might receive up to five traumatic inseminations per feeding which usually lasts for several minutes but can last up to half an hour in some cases (Fedor 2014, How and Lee 2010b, Reinhardt and Siva-Jothy 2007, Usinger 1966).

The life cycle of bed bug has three stages which include eggs, nymphs and adults (Figure 2.2). Under natural condition, a female will begin to produce eggs within 3 days after mating and oviposit eggs individually on rough surfaces such as cloth and wood, where the eggs can occur singly or glued together into clusters (How and Lee 2010b, Miller 2008, Reinhardt and Siva-Jothy 2007). Each female can produce 3–4 eggs every day and may lay 200 to 500 eggs total over her entire lifetime, which can be 2 years or longer (Fedor 2014, Harlan 2006, Miller et al. 2013, Polanco et al. 2011b). The eggs have an elongate-oval shaped with an anterior cap which will be dislodged by the first instar to hatch (Harlan 2006, Khan and Rahman 2012, Miller 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966).

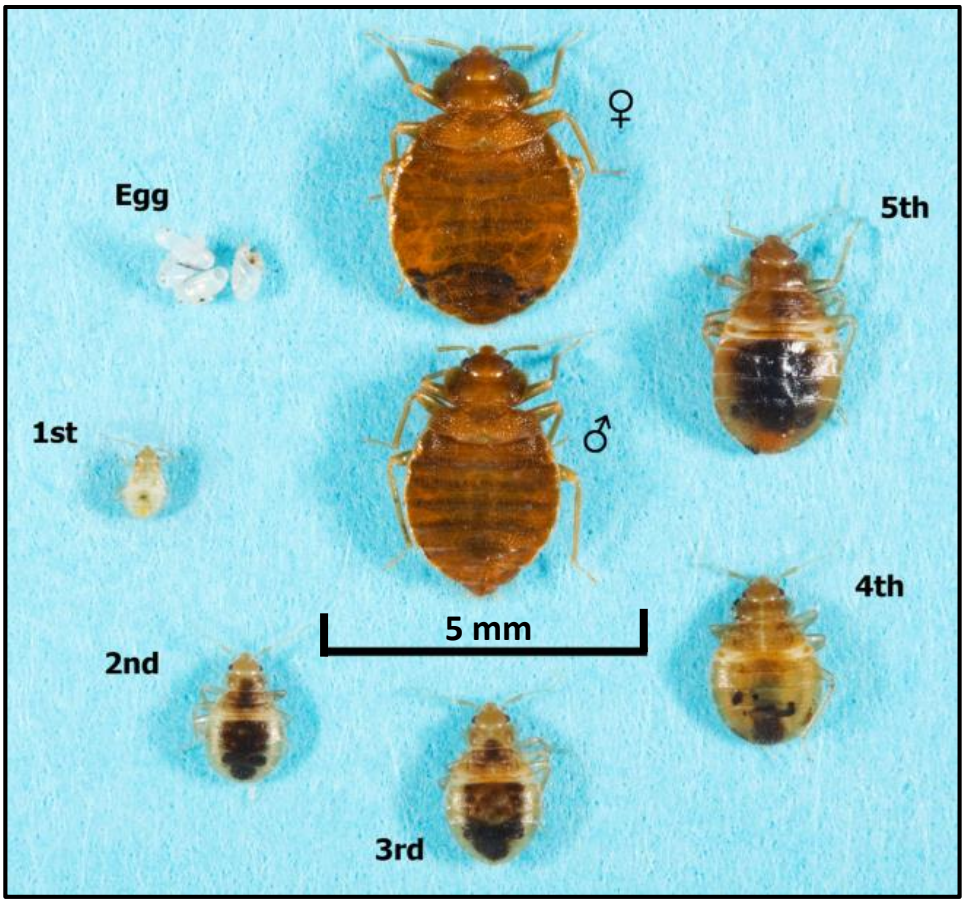

Figure 2.2 The life cycle of bed bugs (Doggett et al. 2012).

Bed bugs reach adulthood by passing through five nymphal stages where the growth and molting of nymphs require at least one blood meal (Fedor 2014, Reinhardt and Siva-Jothy 2007, Usinger 1966). Nymphs are similar in appearance but relatively smaller in size and brighter in color compared to adults (Doggett et al. 2012, Usinger 1966). Each instar will seek blood within 24 hours after molting and all bed bugs are usually stimulated to feed at slightly less than weekly intervals. So bed bugs may emerged as adult as early as five week and can be extended to 4 months or more depending on the ambient temperature and the availability of regular blood meals (Harlan 2006, How and Lee 2010b, Khan and Rahman 2012, Lehane 2005, Miller 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966).

2.2 Tropical Bed Bug, *Cimex hemipterus*

There are three bed bug species that have been associated with human; the common bed bug, *Cimex lectularius* Linnaeus, tropical bed bug, *Cimex hemipterus* Fabricius, and West Africa bed bug, *Leptocimex boueti* Brumpt. *C. lectularius* is distributed widely over temperate region such as the United States, Italy, Kuwait, France, Canada, Australia and the United Kingdom (Akhoundi et al. 2015, Bernardeschi et al. 2013, Durand et al. 2012, El-Azazy et al. 2013, Emmanuel et al. 2014, Giorda et al. 2013, Mumcuoglu and Shalom 2010, Ralph et al. 2013, Wang et al. 2013b, Wu et al. 2014). *C. hemipterus* is found in the warmer tropical and subtropical regions, such as Asia, India, Nigeria, Thailand and Africa (Bandyopadhyay et al. 2015, Okwa and Omoniyi 2010, Tawatsin et al. 2011, Zulaikha et al. 2016), whereas *L. boueti* is found only in West Africa (Weeks et al. 2011). *Leptocimex boueti* also can parasitize bats, while *C. lectularius* and *C. hemipterus* have been associated with a variety of alternative hosts, including bats, birds, and domestic animals (Anderson and Leffler 2008, Reinhardt and Siva-Jothy 2007, Reinhardt et al. 2008, Suwannayod et al. 2010, Usinger 1966, Wang and Wen 2011).

C. hemipterus exhibit similar morphology and biology to *C. lectularius*, but with some difference in their morphology as to differentiate the two species. Entomologists usually look at the pronotum and the shape or hairiness of the dorsal side of the bed bug for species identification (Usinger 1966). In general the pronotum is broad, surrounding the base of the head within the concave anterior margin with the sides of the pronotum extended up around the base of the insect's head (Figure 2.3). The pronotum of *C. hemipterus* is not as broad as *C. lectularius*, and the pronotum margin hairs are curved backward (Khan and Rahman 2012, Pratt and Stojanovich, 1967). The body of *C.*

hemipterus is 1–2 mm longer than *C. lectularius*, with average length of 5.5 mm and average width of 2.5 mm (Khan and Rahman 2012, Usinger 1966). The female spermalege is located at the hind margin of the fifth ventral sternite, and the ectospermalege appears as a transverse dark area (Khan and Rahman 2012, Miller 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966).

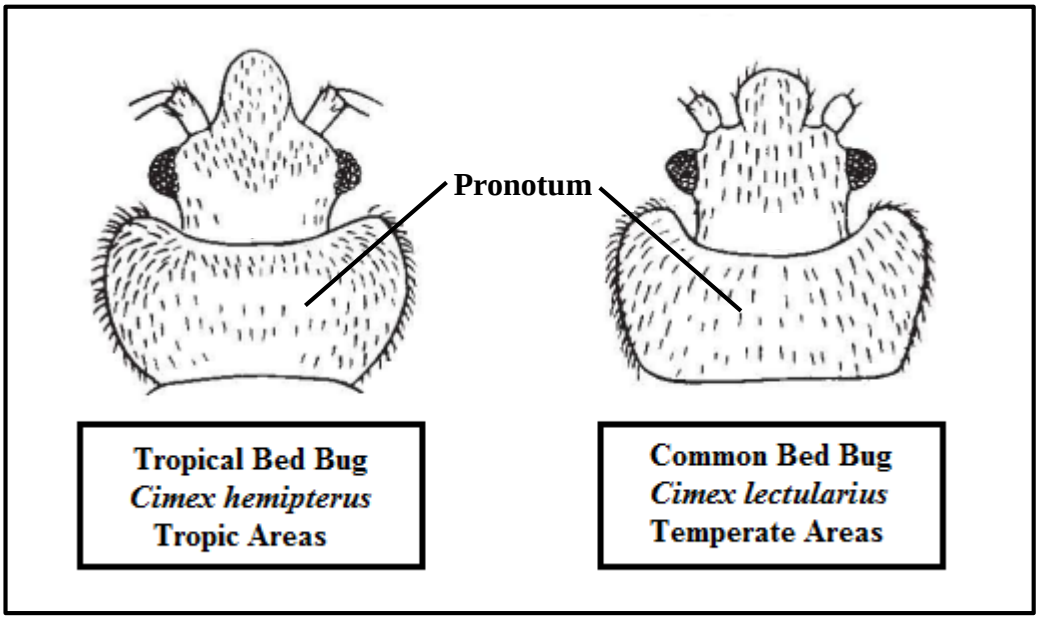

Figure 2.3 Pronotum of bed bugs (Pratt and Stojanovich 1967).

Female bed bugs often deposited their eggs in clusters and attached it to cracks, crevices or rough surfaces near the harborages with some sticky-like substance. Eggs typically hatch in a week to 12 days (How and Lee 2010b, Johnson 1941, Khan and Rahman 2012). The freshly hatched nymph is yellowish colored before feeding, and then turns a reddish color after getting a blood meal (Miller et al. 2013). There are 5 nymphal stages for bed bugs to reach maturity, which usually takes about 32–48 days (How and Lee 2010b, Khan and Rahman 2012, Reinhardt and Siva-Jothy 2007). Each of the five nymph stages of *C. hemipterus* requires about 3–10 minutes to complete the blood feeding

on the host skin while the adults spend relatively more time in feeding, about 10–15 minutes (How and Lee 2010b, Miller et al. 2013, Polanco et al. 2011a, Reinhardt and Siva-Jothy 2007, Suwannayod et al. 2010). Bed bugs have a shorter lifespan when they have adequate available blood sources. Adult bed bugs can survive for up to seven months without blood and have been known to live in empty buildings for up to one year (How and Lee 2010b, Miller 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966).

2.3 Bed Bug Ecology

The ecology of bed bug, especially on population dynamics, dispersal and active movement is probably the aspect that have little progress since Usinger (1966) and Johnson (1941). Bed bugs usually aggregate and live together in their harborages which close in proximity to their hosts. These aggregations contain various life stages, from eggs to adults, dead and live individuals (Siljander et al. 2008, Reinhardt and Siva-Jothy 2007, Usinger 1966). Adults bed bug normally form about one-third of the populations with balance ratio of male and female, thus illustrated that mating competition does not occur within a population (Johnson 1941, Miller et al. 2013, Reinhardt and Siva-Jothy 2007). This characteristic of bed bugs may provide benefits, such as a more favorable environment, reduce exposure to predator, and higher chances of mating (Saenz et al. 2014). They also reported that nymphal development was faster when reared in group rather than in isolation, similar to other gregarious insect species.

Aggregation behavior seem to be maintained by short-range aggregation pheromones emitted by the adults (Johnson 1941, Siljander et al. 2008). Siljander et al. (2008) provided evidence that adult male, virgin adult female, and immature stages of *C.*

lectularius respond to an airborne aggregation pheromone. In addition, aggregations is also stimulated by the mechanical detection of adjacent individuals via antennal mechanoreceptors (Singh et al. 2015, Olson et al. 2009, Olson et al. 2014, Reinhardt and Siva-Jothy 2007). Saenz et al. (2014) suggested that aggregation is important for the moisture maintenance in harborages since bed bugs are sensitive to desiccation, especially during molting (Usinger 1966). Moreover, bed bugs emit substances called alarm pheromones in response to injury, high carbon dioxide concentration or predator's attack, so that the other bed bugs can disperse and hide (Davies et al. 2012, Liedtke et al. 2011, Liu and Liu 2015, Reinhardt and Siva-Jothy 2007).

Bed bugs can travel thousands of kilometers via passive dispersal, through which people might carry the insect in their clothes, luggage or furniture to a new location, unnoticed (Hottel et al. 2015b, Hwang et al. 2005, Reinhardt and Siva-Jothy 2007, Usinger 1966). Places where people travel and stay for a night or two, as hotels, apartments, hospitals, trains, cruise ships, and airports are highly susceptible to bed bug infestations (Bandyopadhyay et al. 2015, Delaunay et al. 2011, Delaunay 2012, How and Lee 2010c, Munoz-Price et al. 2012, Zulaikha et al. 2016). However, recent studies showed that bed bugs can also actively disperse in short distances, from room to adjacent room within a building or between contiguous buildings, mostly through electric wiring or ventilation ducts (Bernardeschi et al. 2013, Booth et al. 2012, Delaunay et al. 2011, How and Lee 2010a, Wang et al. 2010).

Bed bugs move or crawl from one place to another for a various reasons primarily searching for food, mates, or harborage (Goddard et al. 2015, Reinhardt and Siva-Jothy 2007, Usinger 1966). How and Lee (2010a) reported that the fifth instars and adults have

greater movement frequency compare with the other stages with first and second instar have the lowest movement frequency. They also reported that starved bed bugs movement frequency are greater than the blood-fed bed bugs. On the contrary, Goddard et al. (2015) reported that 22% of recently fed bed bugs moved as far as possible in the experimental arena, while only 4% of unfed bed bugs moved to the maximum distance. Movement distance and the time intervals showed a positive relationship, thus indicated that delays in control of bed bugs population will increase and spread the infestation (Booth et al. 2012, Goddard et al. 2015, How and Lee 2010a, Reinhardt and Siva-Jothy 2007).

2.4 Bed Bug Infestations

A survey conducted in infested premises in Malaysia and Singapore by How and Lee (2010c) and another survey in Peninsular Malaysia by Zulaikha et al. (2016) showed that only one species of bed bug was found, the tropical bed bug, *C. hemipterus*. Meanwhile a survey conducted in some dwelling places in Thailand by Suwannayod et al. (2010) showed that even common bed bug, *C. lectularius* can be found in tropical area. *C. lectularius* is known widely spread in United States, Canada, Italy, France, Kuwait, Nigeria and some parts of other country like Chiangmai in Thailand as they prefer temperate area (El-Azazy et al. 2013, Emmanuel et al. 2014, Hwang et al. 2005, Mumcuoglu and Shalom 2010, Omudu and Kuse 2010, Suwannayod et al. 2010).

C. hemipterus is prevalent mostly in Southeast Asia such as Singapore, Vietnam, Indonesia, Taiwan and Malaysia (Bandyopadhyay et al. 2015, How and Lee 2010c, Okwa and Omoniyi 2010, Tawatsin et al. 2011, Zulaikha et al. 2016). While infestations were found to be notably frequent in hotels, bed bugs were increasingly being reported in

homes, apartments, retirement communities, health care facilities, college dormitories, airports, camps and used furniture outlets (Bandyopadhyay et al. 2015, Delaunay 2012, Gbakima et al. 2002, How and Lee 2010c, Hwang et al. 2005, Levy Bencheton et al. 2011, Liu and Pennington-Gray 2015, Liu et al. 2015a and 2015b, Munoz-Price et al. 2012, Ratnapradipa et al. 2011, Wang and Wen 2011, Zulaikha et al. 2016).

Several factors or explanations for the bed bugs resurgence have been suggested by How and Lee (2010b), Moore and Miller (2006) and Wang and Wen (2011). The resurgence can be explained by the increase of resistance level in the insect pest to certain pesticides, development of tourism where people travel from place to place inadvertently help in transporting and distributing bed bug worldwide, socio-economic condition where disposal and second-hand furniture being used in most rental houses, and lack recognition of the existence of bed bugs by people thus allows them to increase in numbers (Levy Bencheton et al. 2011, Liu and Pennington-Gray 2015, Liu et al. 2015a, Liu et al. 2015b). However, it is not clear why only some countries are experiencing bed bugs resurgence. Wang and Wen (2011) found that bed bug infestations especially occurred in crowded living environments or transient environments such as worker dormitories, prisons, and military dormitories and suggested that community-wide bed bug monitoring and control campaigns are necessary for effective control of bed bug infestations.

It is presumed that the crowded environments and frequent occupant turnover were the factors that facilitated the infestations (Reinhardt and Siva-Jothy 2007). Frequent moving and brief stay by the backpackers, immigrants, guest workers, and homeless people may help in transmitting and distribute bed bugs (Gbakima et al. 2002, El-Azazy et al. 2013, Levy Bencheton et al. 2011, Reinhardt and Siva-Jothy 2007). Although passive

dispersal is the main means for bed bugs travelling, active dispersal also plays an important role in infestation that occurred in apartments, hotels and other adjacent buildings as they move from one apartment or hotel room to another via wall voids or electrical conduits (Davies et al. 2012, Delaunay et al. 2011, Goddard et al. 2015). Large-scale movements of military personnel involved in international conflicts have probably all contributed significantly to the re-appearance of bed bugs in many developed countries (Davies et al. 2012). Gounder et al. (2014) reported that households of lower socioeconomic status were more likely to be infested by bed bugs compared to higher income households. They also stated that bed bug infestations directly correlated with the number of children, the number of adult household members, and the number of bedrooms in the house.

One of the major causes of the resurgence of bed bug infestations is the increase of insecticide resistance level that may have been developed by the bed bugs after they had been exposed to a certain insecticide for a long time (Moore and Miller 2006, Romero et al. 2007). Campbell and Miller (2015) conducted an experiment regarding insecticide resistance in eggs and first instars of the *C. lectularius* and concluded that insecticide resistance is expressed early during bed bug development. Recent studies showed that resistance to combination insecticides is present in field populations at levels that should be of concern, and that short-term selection affecting existing variance in susceptibility can quickly increase resistance (Gordon et al. 2014, Gordon et al. 2015, Kilpinen et al. 2011, Mamidala et al. 2011, Perti et al. 2014, Potter 2011, Zhu et al. 2016). Studies by Zhu et al. (2013) revealed that most of the resistance-associated genes functioning in diverse mechanisms are expressed in the epidermal layer of the integument, which could

prevent or slow down the toxin of insecticides from reaching the target sites on nerve cells of bed bugs, where an additional layer of resistance is possible. Understanding all the adaptive strategies of bed bugs regarding insecticide resistance will help in designing new tactics and the most effective and sustainable bed bug control methods.

2.5 Medical Importance of Bed Bug

Since bed bugs feed on blood and always associated with human, it is not surprising that they are often suspected of being vectors of various pathogens and able to transmit diseases (Doggett et al. 2012, Usinger 1966). Review of literatures regarding bed bugs as vectors by Burton (1963) revealed that bed bugs have been suspected in the transmission of 41 human diseases, the agents or causes. Bed bugs have shown capability of carrying the infectious agents of typhus, Q fever, kala-azar, Rocky Mountain spotted fever, anthrax, relapsing fever, plague, tularemia, hepatitis B virus, human immunodeficiency virus (HIV), Chagas disease, and even cancer (Burton 1963, Doggett et al. 2012, Reinhardt and Siva-Jothy 2007). Most of the literature reviewed by Burton (1963) were based on the detection of the pathogen within bed bugs, hence further studies were essential to prove that bed bug is capable of transmitting the agents.

Experimental assessment on HIV were further studied by Jupp and Lyons (1987) and Webb et al. (1989) and concluded that the transmission of HIV virus is unlikely to occur in bed bugs under natural conditions as the survival of the virus in *C. lectularius* and *C. hemipterus* were up to 4 hours and 8 days, respectively. These findings were supported by Silverman et al. (2001) who found that HIV and hepatitis B virus could not replicate inside bed bug's gut, although both virus can persist within the gut for several

weeks. Salazar et al. (2015) reported that the common bed bug may be a competent vector of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. *C. lectularius* and *C. hemipterus* was also found to be able to acquire *Bartonella quintana*, the agent of Trench fever (Angelakis et al. 2013, Leulmi et al. 2015), and maintain it for more than two weeks and capable of transmitting it to their offspring (Leulmi et al. 2015).

The infestation of bed bugs may not transmitted any virus or pathogen, but their need for blood meals has led to a serious public health concern (Eddy and Jones 2011, Lowe and Romney 2011, Munoz-Price et al. 2012, Reinhardt et al. 2009, Thomas et al. 2004). Mouthparts of bed bugs and other blood-sucking insects are evolved and adapted to pierce the skin of their host and inserting extremely fine needlelike stylets into the skin to suck the blood and are withdrawn after feeding (Khan and Rahman 2012, Lehane 2005, Miller 2008, Usinger 1966). During feeding, these insects inject saliva that contains various protein fractions that have anticoagulant properties, which delay the formation of blood clot (Francischetti et al. 2010, Khan and Rahman 2012). The bites of bedbugs is usually painless and usually occurred along the arms and legs as well as on the exposed skin (Doggett et al. 2012, Goddard and de Shazo 2012, Reinhardt et al. 2009).

The most common medical or health concern of bed bugs are the direct cutaneous reactions from the bite. The reaction and manifestations of bed bug bites vary between individuals, from small, hard, swollen welt to severe scratching and dermatological reactions (Plate 2.1). The bite is usually painless, but the salivary fluid that injected together with the bite would cause the skin reactions, although people sensitivity towards bed bug bite is differ with majority of human have allergic reactions to bites, around 70-80% (Eddy and Jones 2011, Goddard et al. 2013, Reinhardt et al. 2009, Serrao et al. 2008).

Typical symptoms include a raised, inflamed, reddish wheal at each bite site (Plate 2.2), which may itch intensely for several days and immediate reactions (Plate 2.3) may appear from 1 to 24 hours after a bite and may last 1–2 days while delayed reactions usually appear 1–3 days or more after a bite and may last 2–5 days (Andres et al. 2015, de Shazo et al. 2012, Goddard et al. 2011, Harlan 2006, Reinhardt and Siva-Jothy 2007, Reinhardt et al. 2009, Thomas et al. 2004).

Plate 2.1 Skin reactions to bed bug bites (Doggett et al. 2012).

Plate 2.2 Wheals caused by bed bug bites (Studdiford et al. 2012).

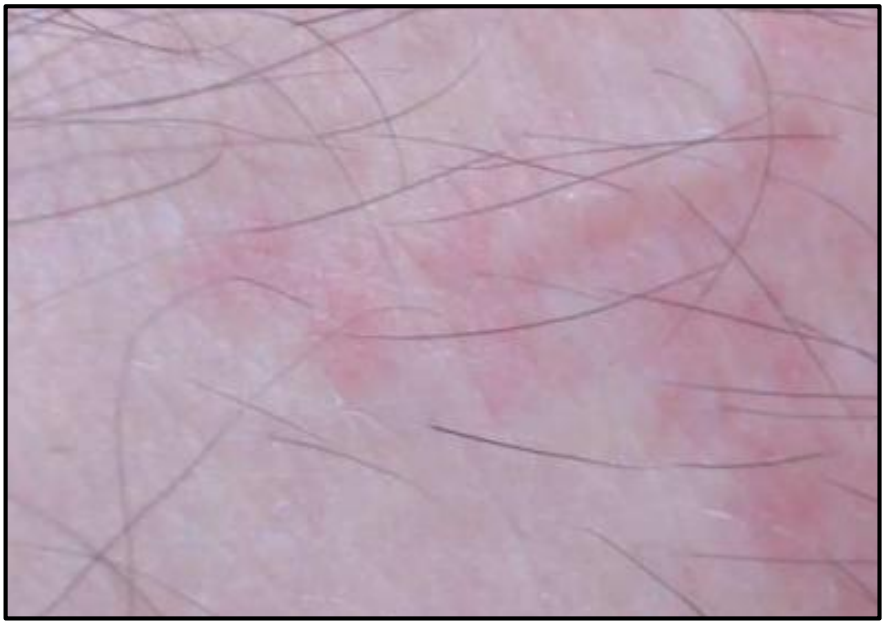

Plate 2.3 Skin lesions manifested 30 minutes after bed bug bites (Goddard and deShazo 2009).

In addition to the physical manifestations of skin reactions, bed bug infestations and bites impact premise's owner mental health, and may lead to significant psychological effects (Hinkle 2010 and 2011, Studdiford et al. 2012, Susser et al. 2012). Frequent feeding can disrupt host's sleep and may result to anxiety, stress, emotional distress and insomnia (Hwang et al. 2005, Ratnapradipa et al. 2011). Patients with multiple bites or a severe cutaneous reaction may develop systemic symptoms, including fever and malaise (Doggett et al. 2012, Reinhardt and Siva-Jothy 2007). Heavy infestation and feeding rates by bed bugs have resulted in significant blood loss and eventual anaemia, especially in malnourished children (Doggett et al. 2012, Goddard and de Shazo 2012, Pritchard and Hwang 2009, Reinhardt et al. 2009). At some extend, bed bug infestations were the likely trigger for the onset of negative psychological state that ultimately led to suicide. Burrows et al. (2013) reported a case of a woman with previous psychiatric morbidity who jumped to her death following repeated bed bug infestations in her apartment.

2.6 Economic Importance of Bed Bugs

Bed bug infestations has been reported to have psychological effects in humans and has a major economic impact, especially in the hospitality and tourism industry (Bernardeschi et al. 2013, Delaunay 2012, Delaunay et al. 2011). Infestation of these blood-sucking insects has caused millions of dollars in losses to cover the cost of service by the pest control operators, infrastructures and furniture replacement, reparation for complains, medical and medicinal cost, and also reputation damages (Heukelbach and Hengge 2009, Hwang et al. 2005, Reinhardt and Siva Jothy 2007). Hwang et al. (2005) reported that the highest number of treatments required per infested location was at the dormitories, hotels, homeless shelters, and rooming houses. The shelters with positive

infestations spent US $150-$15 000 in total for bed bug control efforts which include substantial building repairs such as removing floorboards, replacing carpets, sealing floor cracks, and painting wooden floor (Hwang et al. 2005, Vaidyanathan and Feldlaufer 2013). Meanwhile, various impacts of bed bug infestations in poultry farms include reduced egg value due to bug fecal spots, lower egg production from affected chickens, and loss of productivity via the allergic reactions by workers (Axtell 1999, Doggett et al. 2012, Reinhardt and Siva-Jothy 2007, Rosen et al. 1987).

2.7 Control and Management of Bed Bug

The objective of control and management of bed bug infestations is total elimination, for this insect has a short life cycle but high reproductive ability (Fong et al. 2013, Usinger 1966). A good management and control strategies is crucial to achieve total eradication of bed bug infestation within premises. These strategies must incorporated various measures, which include education, inspection, prevention and control (Bennet et al. 2015, Cooper et al. 2015, Koganemaru and Miller 2013, Singh et al. 2013b). The presence of bed bugs within premises can be determined via inspection either by pest control operators or the owner himself. The easiest inspection is by visual inspection where the signs of bed bug infestation is determined by the presence of blood spots, exoskeletons, empty egg cases, live or dead adult bed bugs, nymphs, and eggs (How and Lee 2010c, Vaidyanathan and Feldlaufer 2013). Other additional inspection tools that can be used to detect the presence of bed bugs include bed bug-detecting canines, intercepting device and trap with various designs (Cooper et al. 2014, Pfiester et al. 2008, Singh et al. 2013a, Wang et al. 2009a and 2009b). Inspection is the first step in controlling bed bug population as this step will determine in designing the control plan (Harlan 2006).

Control methods can be classified into two, physical method where no chemical or minimal quantity of chemical is used for the control, and chemical control which is the most important control measure in battling bed bug infestations. Physical method include bed bug removal through vacuuming or trapping and heat or cold treatment (Eom et al. 2012, Kells and Goblirsch 2011, Naylor and Boase 2010, Olson et al. 2013, Pereira et al. 2009, Rukke et al. 2015, Wang et al. 2011). Additionally, desiccant dust, diatomaceous gels, and silica gels can be used which will damage the cuticle of bed bugs and cause rapid water loss soon after (Benoit et al. 2009, Wang et al. 2009a, Wang et al. 2013a). The addition of alarm pheromone components, carbon dioxide, and a heat generator, increase the chances of bed bugs contact with the desiccant dust and baits (Benoit et al. 2009, Hwang et al. 2005, Wang et al. 2009a, Wang et al. 2012a).

Chemical control, namely residual insecticide treatment is the most important control method used in the efforts to eradicate bed bug infestations (Wang et al. 2014 and 2015). However, bed bugs can hide almost anywhere (Miller 2008, Moore and Miller 2006) making residual insecticide treatment ineffective since bed bugs may not have enough contact with the insecticide, thus not kill them efficiently. Intensive application of chemical insecticide such as deltamethrin, lambda-cyhalothrin, cyfluthrin or phenothrin, or formulations of these pyrethroids with insect growth regulators such as hydropene and/or synergists such as piperonyl butoxide is used by most of the pest control professionals (Davies et al. 2012, Romero et al. 2009). The application concentration of active pyrethroid registered for bed bug control in various commercial products is usually around 1% v/v (Davies et al. 2012). Infestations in places with joining rooms such as hotels and homeless shelters are difficult and costly to eradicate (Hwang et al. 2005). Most

reports also emphasize that it is important to combine insecticide treatments with hygiene measures such as daily laundering of bed linens, vacuuming rooms, and steam cleaning and vacuuming mattresses (Abdul Hafiz and Zulaikha 2015, Fong et al. 2013, Hwang et al. 2005, Jones and Bryant 2012, Wang et al. 2012b, Zhu et al. 2016).

Nowadays, researchers are looking for biological agents that have potential in controlling bed bug population. Ulrich et al. (2014) exposed bed bugs to spores of the entomopathogenic fungus *Metarhizium anisopliae* by feeding, aerosol spray or contact with a treated surface at various humidity level and concluded that the use of *M. anisopliae* as biological control is unlikely. They later reported that bed bug capable of inhibit the fungal growth by emission of (E)-2-hexenal and (E)-2-octenal as defensive secretions (Ulrich et al. 2015). In other study, an oil formulation of conidia of fungus *Beauveria bassiana* were tested against *C. lectularius* on several substrates and bed bugs were found dying most rapidly when exposed to treated jersey knit cotton (Barbarin et al. 2012). Abdul Hafiz et al. (2015) isolated fungal parasites from tropical bed bug and found that *C. hemipterus* are dominantly affected by *Trichoderma harzianum* and three *Aspergillus* sp., *A. nomius, A. tubingensis and A. aculeatus.* Further research in this field could developed appropriate formulations and delivery system of biological agents to battle bed bug infestations in later years.

2.8 Laboratory Rearing of Bed Bug

Maintaining bed bug colonies are crucial to fulfill the need for research on their control and management techniques. However, maintaining large scale of bed bugs in laboratories solely on human hosts has more disadvantages than advantages (Cannet et al.