BIOLOGICAL PARAMETERS, ENVIRONMENTAL EFFECTS, DISPERSAL BEHAVIOUR AND TOXICOLOGICAL STUDIES OF THE TROPICAL BED BUG, *Cimex hemipterus* (FABRICIUS) (HEMIPTERA: CIMICIDAE)

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UNIVERSITI SAINS MALAYSIA

2010

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BY

HOW YEE FATT

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

UNIVERSITI SAINS MALAYSIA

May 2010

ACKNOWLEDGEMENTS

I would like to express my heartfelt thanks to my main supervisor, Professor Dr. Lee Chow Yang, for his meticulous and painstaking guidance, advice and motivation to me in completing this thesis.

I am also grateful to the Dean of School of Biological Sciences, Professor Abu Hassan Ahmad, the coordinator of Vector Control Research Unit, Associate Professor Dr. Zairi Jaal. Thanks are also due to all the staff of Vector Control Research Unit and School of Biological Sciences for providing all the laboratory facilities and assistance especially En, Adanan Che Rus, En. Nasir Hasan, En. Sulaiman Jamaluddin, En. Somasundram a/l Vello, En. Letchimanan a/l Edumban, Pn. Afida Tahir, En. Yusof Roos, En. Shahbudin Shahidan and Pn. Shabariah Ahmed. My candidature as a Ph.D. student in USM would also made possible with financial support from USM Fellowship Scheme and Research Grant from Institute of Postgraduate Studies and Universiti Sains Malaysia.

I would like to register my gratefulness to the following who have been helping me in proof-reading my thesis, listening and discussing the problems that I have encountered throughout my studies, as well as encouraging me - Michael and Janette Lenz (CSIRO Entomology Australia), Xing-Ping Hu (Auburn University), Gaik Hooi, Evan, Ru Yuan, Nellie, Sam, Hui Siang, Kok Boon, Warrin, Veera and Foong Kuan who have been helping me in proof-reading my thesis. Special thank goes to Foong Kuan who had been assisting me in blood-feeding experiments. I also like to thank all the former and present lab members of Urban Entomology Laboratory: Edwin Teo, Pooi Yen, Say Piau, Kean Teik, Boon Hoi, Jing Wen

(Gwen), Lay Tyng, Beng Keok, Tomoki, Kim Fung, Su Yee, Chee Kit, Nadiah, Lily, Yu Chong, Li May, Jia Wei, Ling Hui (Albcerth), Ching Chen, How Jun, Yean Sean, Yoggen, Foong Lee (Chloe), Pui Lai, Ai Hua (Karen), Eng Kooi, Lay Pheng, Steffanie and many others whom I may have missed here.

The assistance provided by following pest control companies during bed bug field sampling and collection is also appreciated: Pest Dynamics Sdn Bhd (Malaysia), NLC General Pest Control (Malaysia), Clean-Environ Pest Management (Singapore), Peter Pest Control Service (Singapore), Legion Pest Control (Singapore), and Alliance Pest Management (Singapore).

Last, but not the least, I wish to thank my beloved family members for their patience, support and love, which have kept me moving ahead in my life.

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ABSTRAK

Tesis ini meliputi kajian mengenai beberapa aspek penting mengenai pepijat, *Cimex hemipterus*, termasuk biologi, keberkesanan persekitaran, perilaku penyebaran dan juga kajian toksikologi dengan menggunakan lapan racun serangga (λ-cyhalothrin, bifenthrin, fenitrothion, fipronil, imidacloprid, indoxacarb, chlorantraniliprole dan DDT). Kebangkitan infestasi pepijat kebelakangan taburan dan pengawalannya juga dibincang.

Sejumlah 54 tempat yang diinfeksi oleh pepijat (Malaysia dan Singapura) telah disarang dari Julai 2005 hingga Desember 2008. Hanya satu spesies yang ditemui, iaitu pepijat tropika, *Cimex hemipterus* (Fabricius). Infektasi pepijat lebih sering didapati berlaku di hotel dan penginapan awam berbanding dengan premis kediaman. Tiga lokasi yang paling digemari pepijat dalam sebuah premis adalah katil, tilam dan kerangkanya (31.09%), kepala katil (30.25%) dan di celah retak pada lantai atau dinding (23.53%).

Parameters biologi pepijat tropika yang dikaji termasuk kesuburan, oviposisi, pengeraman telur, perkembangan nimfa, dan jangka hayat hidup. Dalam keadaan makmal, pepijat menunujukkan kesuburan setinggi 50 telur sepanjang hayat meliputi 11–14 kitar oviposisi. Selepas makan dan mengawan, pepijat betina biasanya mengambil masa 2–3 hari untuk menghasilkan kumpulan telur pertama, dan setiap kitar oviposisi berlanjutan selama 2–7 hari. Julat tempoh pengeraman telur ialah 5–7 hari. Nimfa melalui lima stadia dan mencapai kedewasaan dalam nisbah jantina 1:1. Pepijat

dewasa yang tidak mengawan hidup lebih panjang daripada yang telah mengawan (P<0.05).

Cimex hemipterus bermandirian lebih lama pada suhu rendah (20°C) dan kelembapan tinggi (75–100%). Kemandirian dan kehilangan air dipengaruhi oleh suhu dan kelembapan (termasuk interaksi antara satu sama lain) secara signifikan (P<0.01). Strain dan jantina juga mempengaruhi kemandirian pepijat secara signifikan (P<0.01). Profil kehilangan air menerangkan dengan lebih lanjut mengapa pepijat mula mati selepas kehilangan air 35– 5% badannya.

Peringkat tersebar yang paling aktif adalah instar peringkat kelima, jantan dewasa dan betina dewasa. Pepijat yang lapar menunjukkan frekuensi sebaran yang lebih tinggi berbanding dengan pepijat yang bersarapan darah, kekcuali betina dewasa yang kenyang menunjukkan frekuensi dan jarak pergerakan yang lebih tinggi secara signifikan (P<0.01). Pepijat betina yang kenyang boleh bergerak sehingga >40 m selepas 120 jam.

Daripada kelapan-lapan racun serangga yang diuji terhadap instar peringkat awal, lewat dan imago, didapati bahawa λ-cyhalothrin, bifenthrin, fenitrothion dan fipronil adalah berkesan dalam kawalan pepijat. Individu peringkat lewat menunjukkan LT_{50} tertinggi yang signifikan di antara peringkat hidup yang diuji. Strain atau peringkat pepijat yang berlainan menunjukkan kerintangan yang berbeza terhadap racun serangga yang dikaji. Keberkesanan lambda-cyhalothrin dan fipronil boleh disinergis dengan PBO.

BIOLOGICAL PARAMETERS, ENVIRONMENTAL EFFECTS, DISPERSAL BEHAVIOR, AND TOXICOLOGICAL STUDIES OF THE TROPICAL BED BUG, *Cimex hemipterus* (FABRICIUS) (HEMIPTERA: CIMICIDAE)

ABSTRACT

This thesis describes the biology, environmental effects, and dispersal behavior of the tropical bed bug, *Cimex hemipterus* (Fabricius), as well as the toxicological effects of eight insecticides (lambda-cyhalothrin, bifenthrin, fenitrothion, fipronil, imidacloprid, indoxacarb, chlorantraniliprole and DDT) on this species. The resurgence of bed bug infestations in recent years and infestation management also are discussed.

A total of 54 bed bug infested sites (e.g., hotels, public accommodations, and residential premises) in Malaysia and Singapore were surveyed between July 2005 and December 2008. *C. hemipterus* was the only species found. Bed bug infestations were more common in hotels and public accommodations compared to residential premises. Bedding (31.09%), the headboard (30.25%), and cracks and crevices surrounding the baseboard, wall, or floor (23.53%) were the three most common locations of infestation.

Fecundity, oviposition, egg incubation, nymphal development, and longevity of *C. hemipterus* were the biological parameters studied. Under laboratory conditions, fecundity could reach 50 eggs per lifetime over 11–14 oviposition cycles. After feeding and mating, adult females normally took 2–3 days to produce the first batch of eggs. The oviposition period lasted between 2–7 days before cessation of the oviposition cycle. The egg incubation period usually ranged from 5–7 days before hatching. The nymphs underwent five stadia before becoming adults with a sex ratio of 1:1. Unmated adults lived significantly longer than mated ones ($P<0.05$).

Temperature and relative humidity (RH) both affected bed bug survival. *Cimex hemipterus* survived longest under low temperature (20°C) and high RH (75–100%). Survival and water loss were significantly affected ($P<0.01$) by temperature and RH (either singly or in interaction). Strain and sex also significantly ($P<0.01$) influenced bed bug survival. Water loss profiles further explained the initial death of bed bugs after losing 35–45% of their water content.

The 5th instar, adult males, and adult females were the three most active dispersal stages. Starved insects showed greater dispersal frequency compared to blood-fed individuals, with the exception of adult females. The blood-fed females can move up to >40 m after 120 hours.

Of the eight insecticides tested against early, late, and adult stages of bed bugs, lambda-cyhalothrin, bifenthrin, fenitrothion, and fipronil were efficient in bed bug control. The late stage individuals exhibited the highest significant LT_{50} among the life stages tested. Different strains or life stages exhibited varied tolerance to the tested insecticides. The effectiveness of lambda-cyhalothrin and fipronil can be synergized with piperonyl butoxide (PBO).

CHAPTER ONE

GENERAL INTRODUCTION

Prior to the late 1990s, the bed bug was thought of only as an occasional insect pest. Bed bug infestations generally were considered to be related to hygiene problems and received almost no attention from the public or pest control operators (PCOs). However, the drastic resurgence of bed bug infestations that occurred in the late 1990s awoke the public, and information about bed begs became prevalent in the or news media in many countries, included the United States (Krueger 2000, Miller 2007, Cooper 2006, Potter 2006, Potter et al. 2008), the United Kingdom (Paul and Bates 2000, Boase 2004, Reinhardt and Siva-Jothy 2007), Denmark (Kilpinen et al. 2008), Europe (Owen 2004, Kilpinen et al. 2008), Canada (Myles et al. 2003, Hwang et al. 2005), Italy (Masetti and Bruschi 2007), Australia (Doggett et al. 2003, 2004, Doggett 2006, Doggett and Russell 2008,), and Korea (Lee et al. 2008).

Several hypothetical explanations for the resurgence of bed bug infestations have been suggested (Boase 2008, Doggett 2006, Moore & Miller 2006, Potter 2005, 2006). First, rapid urbanization and globalization could have provided more comfortable habitats (more developed public accommodations and residential houses), suitable microclimates (optimum temperature and humidity), and widespread migration (well-developed transport vehicles and human travel) for bed bugs. Second, there was a dearth of knowledge about bed bugs among the public and PCOs due to the long ‘silent period’ (almost 50 or 60 years) during which bed bugs were only an unnoticed and occasional pest. Third, changes in patterns of insecticide application and insecticide resistance issues increased the difficulty in managing bed bug infestations.

The related bed bug species involved in recent infestation outbreak refers to the common bed bug, *Cimex lectularius* Linnaeus and the tropical bed bug, *Cimex hemipterus* (Fabricius). However, there were relatively less publication and information of *C. hemipterus* compared to that of *C. lectularius* as Wattal and Kalra (1961) also noted that limited information exists about the quantitative bionomics of *C. hemipterus* compared to *C. lectularius*. This has illustrated that some essential biological parameters and knowledges should be studied to fill in the numerous blanks in knowledge before any efficient integrated pest management could be implemented on the tropical bed bug.

Of note with that, this study was designed to examine and evaluate the biological and toxicological parameters of the tropical bed bug, *C. hemipterus*:

a. To initiate the first surveillance study of the status of bed bug infestations in Malaysia and Singapore.

b. To determine the frequency of infestations and the distribution of bed bugs among various infested harborage sites within different types of premises.

c. To examine several essential biological parameters of *C. hemipterus*, namely fecundity, oviposition, nymphal development, and adult longevity, to obtain complete information about its complete life cycle.

d. To examine the survival and water loss of *C. hemipterus* under different temperature and relative humidity (RH) regimes.

e. To study the active dispersal behavior of *C. hemipterus* by evaluating the dispersal frequency and dispersal distance of different life stages and under different feeding regimes.

f. To establish the toxicity profile of selected novel and conventional insecticides against *C. hemipterus*.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Biology of the Bed Bug

Cimicids are temporary and opportunistic blood-feeding ectoparasites that tend to be host specific. For example, bat bugs feed on bats, chicken bugs and swallow bugs feed on poultry and birds, and bed bugs feed on humans. However, cimicids may have alternative hosts that are needed for survival in the absence of the main host (Usinger 1966).

The bed bug has a dorsoventrally flattened body shape, similar to that of cockroaches, that allows it to crawl and hide in narrow cracks and crevices. The head bears pairs of gradually tapering four-segmented antennae and a three-segmented piercing mouthpart. The 11-segmented abdomen has seven pairs of spiracles that are located on segments 2 to 8. Other prominent external morphological features include a broad pronotum, pairs of reduced hemelytral pads (wingless form; this features only appear on adult), and three pairs of slender but well-developed legs for rapid movement (Usinger 1966, Roy and Brown 1970, Askew 1971, Kettle 1984).

The adult bed bug is 4–7 mm long and reddish-brown in colour. The male is distinguished from the female by the asymmetrical pointed posterior part of the abdomen, which is the part of the male sexual organ paramere which covers the real sexual organ (aedegus) of the male. On the other hand, the female has a broader abdomen and bears a notch-like copulatory organ (spermalege) on the ventral side. Bed bugs reach adulthood by passing through five nymphal instar-stages. Nymphs are similar in appearance but relatively smaller in size compared to adults; they also are brighter in colour, have an undeveloped reproductive organ, lack wing pads, have

fewer bristles, have two-segmented tarsi (adults have three-segmented tarsi), and have three prominent dorsal abdominal scent glands. The eggs of bed bugs generally are < 1.1 mm in length and have an elongate-oval shaped with an anterior cap (Usinger 1966, Roy and Brown 1970, Mallis 1990).

The bed bug life cycle has three stages: eggs, nymphs, and adults. Females oviposit eggs individually on rough surfaces, and they can occur singly or glued together into clusters. The first instar dislodges the egg cap to hatch. The growth and moulting of nymphs only progresses if enough blood-feeding occurs. Bed bug mating and oviposition by the female also require blood meals. Thus, all nymphal and adult stages are gregarious and obligate hematophagic ectoparasites: They must ingest blood to survive (Usinger 1966, Roy and Brown 1970, Askew 1971, Mallis 1990).

All cimicids utilize a unique mode of copulation called traumatic insemination, and females of some species have evolved specific adaptations to it. Traumatic insemination describes the copulation process in which the male pierces the female body cavity using its needle-like external genitalia and injects a mass of sperm into the paragenital system and not through the ordinary reproductive tract or opening as most of the insects. The term “traumatic” refers to the integumental wound in the female abdomen after each mating and to the cost of copulation. As an evolutionary adaptation to traumatic insemination, the female has the spermalege as counter-adapt organ, which is divided into ecto- and mesospermalege to reduce the damage of the integumental wound. The mesopermalege also is responsible for sperm selection; it contains phagocyctic haemocytes that may kill low-quality (low-motility) sperm. The regular female reproductive organ (i.e., not the spermalege) and

opening function only in egg laying (Usinger 1966, Stutt and Siva-Jothy 2001, Morrow and Arnqvist 2003, Reinhardt et al. 2003, Siva-Jothy 2006).

2.1.1 *Cimex hemipterus* (Fabricius) (Plate 2.1)

Among the 74 identified cimicids, which include 22 genera and 6 subfamilies (Usinger 1966, Askew 1971), three are categorized as human bed bugs: *Cimex lectularius* Linnaeus, *Cimex hemipterus* Fabricius, and *Leptocimex boueti* Brumpt. *Cimex lectularius* is distributed widely over temperate region such as Canada, the United States, and the United Kingdom. *Cimex hemipterus* is dominant in the warmer tropical and subtropical regions, such as Asia (Malaysia and Singapore), Africa, and the American tropics, whereas *L. boueti* is found only in West Africa. *Leptocimex boueti* also can parasitize bats, and *C. lectularius* and *C. hemipterus* have a wide spectrum of alternative hosts, including bats, chicken, and other mammals (Burden 1966, Usinger 1966, Kettle 1984, Boase 2004).

Both *C. lectularius* and *C. hemipterus* exhibit morphology and biology similar to that described above. However, these two species show some differentiation, which is likely due to the variation of their geographical distribution. Compared to *C. lectularius*, the pronotum of *C. hemipterus* is not as broad or wing-like (it is < 2.5 times wider than its long), and the pronotum margin hairs are curved backwards. The body of *C. hemipterus* generally is longer than that of *C. lectularius* (within 6–7 mm vs. 5–6 mm, respectively). The female spermalege is located at the hind margin of the fifth ventral sternite, and the ectospermalege appears as a transverse dark area (Usinger 1966, Kettle 1984, Mallis 1990).

Plate 2.1 The dorsal view of external morphology of the adult of tropical bed bug, *Cimex hemipterus* (Fabricius). (A) Male (B) Female

Each of the five nymph stages of *C. hemipterus* requires about 3–10 minutes to complete the blood feeding at the host skin and 0.4–7.6 mg of blood volume as food while the adults spend relatively more time in feeding, about 10 – 15 minutes. After feeding well, the male eagerly and aggressively seeks for a fed female. Stationary copulation takes about 20 seconds, but up to 100 seconds are required when the female is not in the static mode (Wattal and Kalra 1961). The authors also showed that *C. hemipterus* most preferred humans as their blood source, as it produced the highest yield of oviposited eggs, followed by chickens, rabbits, and rats. In contrast, host-preference tests conducted by DeMellion and Golberg (1947) and Johnson (1937) on *C. lectularius* showed that nymphs exhibited the largest percentage of successful growth when fed on animal blood rather than human blood.

Most biological studies of *C. hemipterus* were conducted before World War II (Patton and Cragg 1913, Dunn 1924, Mellanby 1935, Geisthardt 1937, Hase 1931 cited in Usinger 1966). Omori (1941) conducted an extensive biological study and Wattal and Kalra (1961) made laboratory observations, but otherwise there is a dearth of information about the biology of *C. hemipterus*. Wattal and Kalra (1961) also noted that limited information exists about the quantitative bionomics of *C. hemipterus* compared to *C. lectularius*. However, the resurgence of bed bug infestations that began in the late 1990s (Boase 2008) and drastically increased in the 2000s (Doggett and Russell 2008) has illustrated that some essential biological parameters should be studied to fill in the numerous gaps in our knowledge. Of note is the fact that the increase in bed bug infestations in the ASEAN region (Malaysia and Singapore) has been caused solely by *C. hemipterus*, and infestations by this species have also been reported in Australia.

2.2 The Resurgence of Bed bug Infestations

The resurgence of bed bug infestations has been described as a global pattern that involves many countries (Reinhardt and Siva-Jothy 2007). Increasing numbers of bed bug infestations have been reported in the United States (Krueger 2000, Cooper 2006, Miller 2007, Potter et al. 2008), the United Kingdom (Paul and Bates 2000, Boase 2004, Reinhardt and Siva-Jothy 2007), Denmark (Kilpinen et al. 2008), Europe (Owen 2004, Kilpinen et al. 2008), Canada (Myles et al. 2003, Hwang et al. 2005), Italy (Masetti and Bruschi 2007), Australia Doggett et al. 2003, 2004, Doggett 2006, Doggett and Russell 2008), and Korea (Lee et al. 2008).

Although both *C. hemipterus* and *C. lectularius* tend to follow their dominant and allopatric territory distribution, some exceptions exist in which the two species co-occur or found in the same country. Wattal and Kalra (1961) reported co-occurrence in the hilly regions of India; Newbery (1988, 1989, 1990) and Walpole and Newberry (1988) reported the discovery of both species in a domestic infestation in northern KwaZulu, South Africa; and the collected insect samples from Australia suggested the intrusion of both species (Doggett et al. 2003, 2004). In addition, when both *C. hemipterus* and *C. lectularius* have occasionally occurred in sympatry, interspecific mating could occur, as a hybrid of the two species has been reported (Newberry 1988, 1989, Walpole and Newberry 1988).

Explanations (Potter 2005, 2006, Doggett 2006, Moore & Miller 2006, Boase 2008) for the resurgence of bed bug infestations can be categorized into three large groups of issues: social, environmental, and pest control. The environmental issues involve global warming and central heating; increased temperature within the bed bug's optimum range could improve the reproductive rate and numbers, or even

nymph growth, thereby increasing the spread and severity of infestations. The ubiquitous application of temperature-control devices, such as air conditioners, may create a comfortable zone for both humans and bed bugs.

The social issues involve human activity, which can help disperse bed bugs from place to place. For example, trading of infested second-hand items or global travel and migration can accidentally transport bed bugs from hotel to hotel or from hotel to home. Advanced transport systems (Whitfield 1939) and globalization can spread bed bugs to anywhere humans go. The increasing human population gradually leads to overcrowding in urbanized areas, which also maximizes the food source for bed bugs. Public awareness about bed bug infestations remains low, as infestations have not been a serious problem for almost 60 years.

Pest control operators and entomologists also may lack adequate knowledge about how to handle some urban and medical pest infestations (Matthews 2008), and bed bug infestation is a good example of this problem. Changes in patterns of insecticide application also may have contributed to the spread of bed bug infestations. The baiting method of controlling cockroaches is not efficient for bed bug management. Thus, more studies and evaluations on various insecticides are needed to determine insecticides that can be used effectively to manage bed bug infestations.

Insecticide resistance is one of the major causes of the resurgence of bed bug infestations. Insects may develop resistance mechanisms after long-term exposure to a particular insecticide, which could render it ineffective for use in pest control. Some researchers reported that bed bugs developed DDT (dichlorodiphenyltrichloroethane) or analogous insecticide (example, γ-BHC)

resistance when DDT was extensively used as a control chemical soon after World War II (Johnson and Hill 1948 cited in Usinger 1966, Brown 1958, Busvine 1958, Lofgren et al. 1958, Gratz 1959, Mallis and Miller 1963, Cha et al. 1970). However, the use of DDT was banned due to its persistenance in the environment.

Pyrethroids are a common group of active ingredients in home-use or pest control products up to date. This group is commonly used for bed bug management because infestations are always associated with human living or resting places, thus safe insecticides or insecticides at low concentrations must be used. However, reports of pyrethroid resistance exist (Myamba et al. 2002, Moore and Miller 2006, Karunaratne et al. 2007, Romero et al. 2007, Boase 2008, Kilpinen et al. 2008). To overcome these resistance problems, alternative insecticides (Barile et al. 2008, Turner and Brigham 2008) that use synergistic chemicals (Romero et al. 2009a) or other efficient control measures (Miller and Fisher 2008, Roberto et al. 2009b) should be emphasized and investigated.

2.3 Importance of bed bugs

Many people suspect that bed bugs, as blood-feeding insects, have great potential to transmit blood-borne diseases, such as filariasis, Kala-azar, yellow fever, relapsing fever, plague, Hepatitis B and AIDS (Burton 1963, Nelson 1963, Ogston et al. 1979, Jupp and Lyons 1987, Webb et al. 1989, Jupp et al. 1991, Blow et al. 2001). However, although bed bugs have been proved capable of carrying many pathogens or infectious particles (Strand 1977), no reports of natural cases of disease transmission by bed bugs exist (Usinger 1966, Reinhardt and Siva-Jothy 2007, Potter et al. 2008, Stucki and Ludwig 2008, Goddard and deShazo 2009, Heymann 2009).

Bed bug bites can cause secondary infection if they are scratched and accidentally exposed to infectious pathogen accidentally. Known secondary infections include folliculitis, cellulitis, and eczematoid dermatitis (Allington and Allington 1954, Feingold et al. 1968, Crissey 1981, Goddard and deShazo 2009).

Bed bug bites caused a cutaneous reaction in humans, and the severity of the reaction depends on individual sensitivity, exposure time, and numbers of bites (Reinhardt et al. 2009). Reinhardt et al. (2009a) reported that almost 80% of their study population was sensitive to bed bug bites, and they concluded that the prevalence of bed bug bite sensitivity would increase if infestations spread.

Individuals with hyposensitivity to bed bug bites develop only a hemorrhagic punctum on the skin and experience few delayed reactions. In contrast, individuals with hypersensitivity to the bites may experience anaphylactic-like reactions, including pruritic macupapular rashes, erythematous lesions, itchiness, urticaria, inflammation, and bullous rashes (Cestari and Martignago 2005, Stucki and Ludwig 2008, Goddard and deShazo 2009, Heukelbach and Hengge 2009, Heymann 2009). High numbers of bites can lead to more severe reactions, such as blister papules or systemic hypersensitivity (Thomas et al. 2004, Heymann 2009) and loss of haemoglobin or iron deficiency (Venkatachalam and Belavady 1962). Some reports have suggested that the bed bug bite reaction is probably IgE mediated and caused by the human immunological responses to the salivary proteins of the bed bug, such as nitrophorin, factor X, and a 40-kDa apyrase-like nucleotide-binding enzyme (Valenzuela et al. 1996, 1998, Valenzuela and Ribeiro 1998, Goddard and deShazo 2009, Heymann 2009, Klotz et al. 2009).

Bed bug bites and infestations can also lead to a psychological phobia in some people. Delusory parasitosis describes the condition in which a person experiences the delusional belief that they are still surrounded by the insect (example: bed bugs) even through the infestation has been eliminated. This may cause some forms of psychosis, such as formication and insomnia (Hinkle 2000, Lee et al. 2008, Heukelbach and Hengge 2009).

Other than medical importance, bed bug infestation also plays a role in contributing to economic losses, especially in the hospitality and tourism industry (Doggett 2006, Doggett and Russell 2008, Heukelbach and Hengge 2009). The multimillion dollars in losses involved pest control costs, replacement of infested infrastructures, reparation for complaints, medical costs, and also reputation damage (Coleman 2005, Hwang et al. 2005, Doggett 2006, Miller 2007, Shavell 2007, Doggett and Russell 2008, Potter et al. 2008, Heukelbach and Hengge 2009). For example, in San Francisco, CA, USA, professional bed bug management reportedly costs $2000 per infested unit, and some hotels had to pay up to $60,000 to control an outbreak (Miller 2007). Coleman (2005) and Shavell (2007) reported that a victim of bed bug bites won a lawsuit against a motel and was awarded $186,000 in punitive damages. In Australia, the resurgence of bed bug infestations has had an economic cost of an estimated $AUS 100 million (Doggett and Russell 2008).

2.4 Environmental Effects on Bed Bugs: Temperature and Humidity Studies

Edney (1957) stated that the relationship between water and terrestrial arthropods (insects in particular) is an important component of ecological study. This

relationship involves water loss, water gain, and the effects of water on body temperature and thermal resistance. Insects are poikilothermic animals (Romoser and Stoffolano 1998) and because of that, environmental factors play an essential role in the insect–water relationship. Among the critical extrinsic factors that affect biological parameters such as longevity and development, temperature and humidity are of great interest to many entomologists.

The literature contains few articles about temperature, humidity, and bed bugs. A few studies were conducted for *C. lectularius* (Hase 1930 in Usinger 1966, Mellanby 1935, Kemper 1936, Johnson 1940a, 1940b, 1941, Omori 1941, Benoit et al. 2007, Roberto et al. 2009) and *C. hemipterus* (Hase 1930 in Usinger 1966, Mellanby 1935, Omori 1941). Usinger (1966) reported that temperature is the most important of the various extrinsic factors, as it has extensive influence on all aspects of bed bug activities.

Omori (1941) found that *C. lectularius* and *C. hemipterus* preferred aggregation and clustering within 28–29°C and 32–33°C, respectively. These two species exhibited different temperature tolerances, and thus their biology and/or physiology may also differ when exposed to various temperatures. When the lower thermal lethal limit was investigated, *C. hemipterus* was found to be less resistant to cold temperature (up to 10°C), whereas *C. lectularius* could survive in temperatures as low as 6°C (Usinger 1966). For the upper thermal lethal limit, both species died within 1 hour at 44°C and within 24 hours at 40°C (Mellanby 1935). Generally, bed bugs will survive for a shorter length of time at higher temperature and a longer period at lower temperature (Johnson 1941, Omori 1941, Usinger 1966).

Johnson (1941), Kemper (1936), and Omori (1941) concluded that humidity was another important environmental factor affecting bed bugs. Different humidity levels were found to affect bed bug fertility, fitness, longevity, and even development period. Generally, bed bugs do not prefer low humidity (Johnson 1940b, 1941). Johnson (1941) reported that the favourable humidity range may be between 50% and 80% RH. Omori (1941) reported that extremely high humidity can contribute to shortened longevity of the adult bed bug. The lower survival rate of bed bugs in excessively humid conditions may be related to a variety of factors, including uptake of saturated water vapor due to greater critical equilibrium activity (Benoit et al. 2007) and vulnerability to massive growth of viruses or bacteria under highly humid conditions (Romoser and Stoffolano 1998).

In terms of details about the tropical bed bug, Omori (1941) found that the *C. hemipterus* egg incubation period was as long as 17.1 days at 20°C and 83% RH. Mated females could live up to 33 days at 33°C under 70–80% RH, whereas unmated females showed longer survival at 33°C (107–124 days). Omori (1941) also reported that mated males could survive relatively longer than mated females, for example 24–42 days longer at the two above-mentioned temperatures and RH. However, the author did not report the survival of unmated male bugs, which could be an interesting subject as any detrimental mating effect to males instead of females.

Omori (1941) conducted an outdoor study of longevity of both bed bug species in different seasons (winter, summer, and spring). The longevity exhibited variation that was related to the different temperature and humidity values that characterized the seasonal climate. Omori also compared longevity between species when they were exposed to various constant temperatures (15, 18, 22, 27, 30, and 33°C) at 75–98% RH. Johnson (1940b) reported that *C. lectularius* longevity was

indirectly related to the water loss rate under different humidity and temperature conditions. Overall, the results suggest that temperature and humidity affect not only individual bed bug longevity but also interactions among bed bugs.

Benoit et al.'s (2007) study of the *C. lectularius* water balance resistance profile explained how bed bugs tolerate water stress by water conservation, aggregation, and quiescence behavior. Mating effect, strain, sex, and exposure time are factors that need to be considered when examining the effects of temperature and humidity on bed bugs; Johnson (1940b) also considered host blood type and virginity of female bugs.

2.5 Dispersal behavior

Dispersal behavior of bed bugs has been studied extensively due to the importance of tracking and preventing the spread of infestations. However, numerous questions remain unanswered on bed bug dispersal behavior; in particular, the reason for the resurgence of infestations in the 1990s, or the distance a bed bug can disperse itself. Generally, bed bug dispersion can be categorized into two main routes: active dispersal and passive dispersal (Reinhardt and Siva-Jothy 2007).

Active dispersal behavior refers to self-movement from a natural harborage that is initiated by disturbance (e.g. treatment by pest control operators) (Romero et al. 2009b), stress (hunger, temperature, mating avoidance, alarm pheromones, etc.) (Johnson 1941, Griffiths 1980, Bell 1990, Pfiester et al. 2009a), overgrowth of the population, or aggregation (Wertheim et al. 2005). Active dispersion represents one of the main mechanisms for the spread of infestations over short distances, especially inside a room and within contiguous rooms. However, active dispersion requires a lot

of energy, and Mellanby (1938) reported that bed bugs that explored over long distances often starved to death. As a consequence, under optimal conditions (presence of host and no other external or internal forces apply) bed bugs generally remain motionless in their harborage close to the host (Johnson 1941) and only search over short distances for a blood host (Usinger 1966).

Some studies have shown that females are more likely than males or nymphs to actively disperse (Mellanby 1939b, Johnson 1941). All stages of nymphs are inactive stages and do not actively disperse; they prefer to aggregate in their harborage due to air-borne and contact pheromones. In contrast, the alarm pheromone would be the chemical substance that would promote and activate the active dispersal movement of bed bugs (Benoit et al. 2009).

Passive dispersal behavior refers to the more common spreading route of bed bug infestations that involves various host-associated belongings and activities. Bed bugs can be spread passively in clothing, luggage, belongings, and furniture. This mechanism explains the long-distance dispersion of bed bugs, as these insects are wingless and thus cannot fly. Whitfield (1939) reported that frequent travelling via various type of transportation, including car, van, train, ship, and airplane, may encourage the dispersion of bed bugs from country to country. Globalisation could become the largest pathway leading to a worldwide distribution of bed bugs.

Kells (2006) stated that modern society provides a convenient "hitchhike" system by which bed bugs can move between various potential harborage and nesting sites. He further classified harborage sites into two groups: temporary habitation sites (lodging establishments, such as hotels) and permanent nesting sites (long-term residence premises, such as homes and apartments). Other cimicids have

different dispersion mechanisms. For example, the swallow bug, *Oeciacus* sp., can be carried by birds, and the bat bug, *Cimex pilosellus* (Horvath), can attach to bats and be dispersed when the host establishes a new nest site (Usinger 1966, Dick et al. 2003).

2.6 Management of Bed bug Infestations

Bed bugs have a short life cycle and high reproductive ability, so the goal of bed bug management is simply bed bug elimination. Boase (2008) and Doggett (2006) reported that the persistence of bed bug infestations in public premises is caused by failure to achieve full eradication of the insects. Thus, a comprehensive strategy for eradication of an infestation within premise must be well planned and must integrate various measures, including inspection, monitoring, control, prevention, and education.

2.6.1 Inspection and Monitoring

Inspection and monitoring are two important procedures in managing bed bug infestations because they help detect the presence of bed bugs within a premise and assist in locating the harborage sites. Inspection and monitoring also provide live bed bug samples for species identification, which is important for designing the control plan (Doggett 2006, Harlan 2006). Inspections require manual inspection by well-trained workers with torch light and fine forceps. Other additional study tools include bed bug-detecting canines (Pfiester et al. 2008), a bed bug intercepting device (Wang et al. 2009a), a pitfall trap baited with carbon dioxide, heat, and chemical lures

(Wang et al. 2009b). Up to date, however, most inspections still rely on manual inspection by a human due to cost, availability, and other issues.

2.6.2 Education and Prevention

Education about inspection, monitoring, biology, and management of bed bug infestations is current widely available worldwide, in the form of seminars (Barile et al. 2008, Boase 2008, Doggett and Russell 2008, Kilpinen et al. 2008, Miller and Fisher 2008, Naylor et al. 2008, Potter et al. 2008, Turner and Brigham 2008), professional training and talks (How 2007, 2008), handbooks and publications (Doggett 2006, Pinto et al. 2007), and internet websites (www.bedbugcentral.com, Bed Bug Central TV in Anonymous 2009). These activities prominently point out that the cooperation of the public and pest control companies against bed bug infestations is an important element in enhancing the efficacy of pest management strategies.

Prevention of bed bug infestations can also occur by training hoteliers, housekeepers, and the public to detect the presence of bed bugs and to conduct regular monitoring. Infestation prevention measures include improved hygiene maintenance, bed design (fewer cracks and crevices, metal frames), and room design (all possible cracks and crevices are well sealed, not using parquet or carpeted floor, and examination of new or second-hand furniture) (Doggett 2006).

2.6.3 Physical and Chemical Control

Bed bug infestation control methods can generally be categorized into two groups: physical and chemical. Physical control methods do not use any chemicals or minimize the quantity as much as possible. These methods include bed bug removal through vacuuming (Doggett 2006) or trapping (Doggett 2006, Wang et al. 2009a, 2009b) and heat or cold treatment. In the latter, the objective is to kill the bed bugs using knowledge of their critical upper and lower temperature limits (Doggett 2006, Roberto et al. 2009). Desiccant dust, diatomaceous gels, and silica gels can be used to cause cuticular damage followed by rapid water loss (Benoit et al. 2009, Wang et al. 2009a).

Some physical devices can be modified by the addition of a low toxicity chemical as a synergist to improve their efficacy. For example, the addition of alarm pheromone components can prompt the movement of bed bugs and enhance the chances that they will contact the desiccant dust (Benoit et al. 2009). The addition of carbon dioxide, a heat generator, or aggregation pheromone added to bait in a pitfall trap also can be used (Wang et al. 2009b). However, some traditional physical methods, such as wrapping the mattress in black plastic and exposing it to direct sunlight, may not provided efficient bed bug control (Doggett et al. 2006).

Chemical control especially the residual insecticide treatment, is the most important control measure in battling bed bug infestations. Due to their cryptic nature, bed bugs are able to hide almost anywhere (in particular in obscure cracks and crevices) (Usinger 1966, Boase 2004, Potter et al. 2008, Moore and Miller 2009). Thus, spraying may not last long enough or have enough contact with the bed bugs to kill them. Bed bugs infest human resting places or other places in the home, and this

proximity to humans restricts the use of many classes of chemical. Therefore, most of the currently registered insecticide products used for bed bug control are either natural pyrethrins or synthetic pyrethroids (Doggett 2006, Barile et al. 2008, Doggett and Russell 2008, Potter et al. 2008, Turner and Brigham 2008, Moore and Miller 2009).

2.7 Insecticides and Their Mode of Action

In the present toxicological study, the following insecticides were tested for their ability to kill bed bugs: organochlorine (DDT), organophosphate (fenitrothion), pyrethroids (bifenthrin, lambda-cyhalothrin), chloronicotinyl (imidacloprid), phenypyrazole (fipronil), oxadiazine (indoxacarb), and anthranilic diamide (chlorantraniliprole).

2.7.1 Organochlorine (DDT)

Insecticides prior to the 1940s were mostly inorganic chemicals (e.g., arsenic). The use of organic insecticides increased after World War II. Organochlorines were the first of the synthetic organic insecticides, and they can be classified into two groups that differ in their toxicological mode of action: 1) DDT and its analogues (e.g., γ-BHC) and 2) cyclodiene (e.g., deldrin). DDT causes hyperexcitation or repetitive discharges of impulse along the voltage-sensitive sodium channels of nerves (Narahashi 1971, Matsumura 1985, Narahashi et al. 2007). After World War II, DDT was widely used to battle various disease vector insects, such as mosquitoes, houseflies, and other biting flies. Bed bugs were also controlled by DDT at that time,

but they subsequently developed DDT resistance (Johnson and Hill 1948 cited in Usinger 1966, Brown 1958, Busvine 1958, Lofgren et al. 1958, Gratz 1959, Mallis and Miller 1963, Cha et al. 1970). Recently, prolonged DDT resistance in bed bug populations was detected (Karunaratne et al. 2007, Steelman et al. 2008).

2.7.2 Organophosphate (fenitrothion)

In the 1950s, malathion became the first marketed organophosphate insecticide. Organophosphates and their metabolites are potent inhibitors of esterases. Acetylcholinesterase is a widely distributed enzyme that hydrolizes the neurotransmitter acetylcholine into acetate and choline at the nerve synapse. Inhibition of acetylcholinesterase results in local accumulation of acetylcholine in cholinergic synapses and leads to hypercholinergic activity. Subsequently, signs and symptoms of intoxication appear, including rapid twitching of voluntary muscles and paralysis. Most organophosphates require metabolic bioactivation to become effective against acetylcholinesterase (O'Brien 1967, Fest and Schmidt 1982, Chambers et al. 2010). Fenitrothion [O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] showed broad-spectrum activity as a contact and stomach insecticide (Fest and Schmidt 1982) and was categorized as a moderately hazardous organophosphate, as was chlorpyrifos (Chambers et al. 2010).

2.7.3 Pyrethroids (bifenthrin, lambda-cyhalothrin)

Pyrethroids constitute a class of synthetic insecticides with low hazard to mammals. Pyrethroids are derived from the structure of pyrethrum, which is an

effective natural insecticide. Compared to pyrethrum, pyrethroids have relatively high photostability and last longer, thereby providing effective residual activity against insect pests. In the late 1970s, pyrethroids began to be marketed commercially as a replacement for organochlorine and organophosphate insecticides in agricultural use. Pyrethroids gradually became the most common insecticides found in household insecticide products for both medical and urban insect pest control (Soderlund 2010). The mode of action of pyrethroids is similar to that of DDT: They attack and disrupt voltage-sensitive sodium channels, which results in hyperexcitation or repetitive discharge in nerves (Narahashi 1971, Soderlund and Knipple 2003, Narahashi et al. 2007). Piperonyl butoxide (PBO), which inhibits the mixed function oxidase (MFO) system of insects, commonly is used with pyrethroids as a synergist for more effective insect control (Casida 1970, Osmitiz 2010).

The pyrethroids can alternatively be segregated into two subgroups—Type I (non-cyano pyrethroids) and Type II (alpha-cyano pyrethroids)—based on the intoxication syndrome produced in mammals. Type I commonly produces T-syndrome (tremors) and Type II commonly produces CS syndrome (choreoathetosis with salivation) (Lawrence and Casida 1982, Breckenridge et al. 2009, Soderlund 2010). These two subgroups can also be divided according to their electrophysiological profile, chemical structures and the symptoms they produce in poisoned insects, Gammon et al. 1981, Soderlund 2010). Bifenthrin [2-Methylbiphenyl-3-ylmethyl(*Z*)-(1*RS*)-*cis*-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is a Type I pyrethroid, and lambda-cyhalothrin[(*RS*)-α-cyano-3-phenoxy-benzyl(*Z*)-(1*R*)-*cis*-3-(2-chloro3,3,3trifluoropropenyl)2,2dimethylcyclopropanecarboxylate] is a Type II pyrethroid (Breckenridge et al. 2009, Soderlund 2010). Pyrethroid resistance has

been reported in bed bugs (Myamba et al. 2002, Moore and Miller 2006, Karunaratne et al. 2007, Romero et al. 2007, 2009a, Boase 2008, Kilpinen et al. 2008, Steelman et al. 2008, Yoon et al. 2008).

2.7.4 Neonicotinoid/Chloronicotinyl (imidacloprid)

Neonicotinoid insecticides, which are also referred as chloronicotinyls, represent a new generation of broad-spectrum nicotine-related insecticides with excellent systemic, oral, and contact activity. Their mode of action is based on the antagonism on post-synaptic nicotinic receptors. Nicotinic acetylcholine receptors (nAChRs) in the insect nervous system are the principal sites of action (Liu et al. 1993, Buckingham et al. 1997, Matsuda et al. 2001, Sheets 2010). Compared to nicotinoids, neonicotinoids are selectively toxic to insects and less toxic to vertebrate species (Tomizawa and Yamamoto 1992). Imidacloprid [1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine] is the first representative registered neonicotinoid insecticide , and it has become a commercially important neonicotinoid insecticide (Matsuda et al. 2001, Sheets 2010).

2.7.5 Phenylpyrazole (fipronil)

Phenylpyrazoles act as a GABA-gated chloride channel blockers, same as the cyclodiene insecticide in organochlorine group. GABA (γ-aminobutyric acid) receptor is contrast to the function of chlolinesterase receptor, it is responsible in the binding sites for the inhibitory neurotransmitter but not for the excitatory neurotransmitter (Osborne 1996, Zhao et al. 2003, Ikeda et al. 2004). Fipronil is a

phenypyrazole insecticide that was developed in the mid-1990s. It exhibits high selective toxicity on insects but not on mammals (Zhao et al. 2003, Ikeda et al. 2004, Narahashi et al. 2007). Fipronil and its metabolite, fipronil-sulfone, not only inhibit transmission at GABA receptors but also target the glutamate-activated chloride channels (GluCls) at the central synapse of insects (Ikeda et al. 2004, Zhao et al. 2005, Narahashi et al. 2007). Thus, both the parent and metabolite molecule of fipronil are toxic to insects. The addition of PBO (piperonyl butoxide) as a synergist to fipronil has produced varied results for different insects (Scott and Wen 1997, Valles et al. 1997, Scharf and Siegfried 1999, Wen and Scott 1999, Liu and Yue 2000, Wu et al. 2004, 2007, Kang et al. 2006, Li et al. 2007).

2.7.6 Oxadiazine (indoxacarb)

Development of the pyrazoline-type insecticides has generated a new branch of pyrazoline insecticides called oxadiazines, which have an oxadiazine ring at the centre (Silver and Soderlund 2005). Indoxacarb is an oxadiazine that blocks the insect voltage-gated sodium channel after metabolite bioactivation into the N-decarbomethoxyllated metabolite (Wing et al. 2000). Indoxacarb has been used against agricultural pests (Lepidoptera, Homoptera, Coleoptera) (Wing et al. 2000) and urban pests, including house flies (Shono et al. 2004) and cockroaches (Buczkowski et al. 2008).

2.7.7 Anthranilic diamide (chlorantraniliprole)

Diamide insecticides are one of the novel insecticides that is effective against insects and its characteristic of low hazard has minimal toxicity to mammals.