

**SUSCEPTIBILITY OF HUMAN BED BUGS (*Cimex hemipterus*) TO SELECTED
INSECTICIDE PRODUCTS IN THREE EDUCATIONAL INSTITUTIONS
IN ACCRA METROPOLIS, GHANA**

BY

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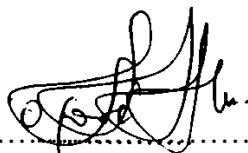
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DECLARATION

I, Eva Duoduaa Ofori, hereby certify that this thesis is the outcome of a research undertaken by me, towards the award of Master of Philosophy in Entomology in the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Legon. This thesis has not been submitted elsewhere either in part or full, for any other degree and all references to work of other people have been duly acknowledged.



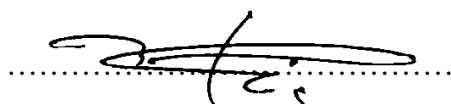
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DEDICATION

I dedicate this work to my beloved mother, Mrs. Margaret Sodoke.

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TABLE OF CONTENT

DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENT	iv
LIST OF FIGURES	vii
LIST OF TABLES	viii
LIST OF APPENDICES	ix
LIST OF ABBREVIATIONS	x
ABSTRACT	xii
CHAPTER ONE	1
1.0 INTRODUCTION	1
1.1 Study Background	1
1.2 Justification	3
1.3 Objectives	4
1.3.1 Main Objectives	4
1.3.2 Specific Objectives	4
CHAPTER TWO	5
2.0 LITERATURE REVIEW	5
2.1 Origins of Bed Bugs	5
2.2 Biology of Bed Bugs	6
2.2.1 Life Cycle of Bed Bugs	8
2.3 Global Distribution of Bed Bugs	10
2.4 Health Impacts of Bed Bugs	10
2.5 Social Impacts of Bed Bugs	13
2.6 Identification of Bed Bug Infestation	14
2.6.1 Detection Methods	15
2.6.1.1 Basic tools	15
2.6.1.2 Active and Passive detection systems	15
2.7 Prevention and Control of Bed Bug Infestation	16
2.7.1 Starvation	17
2.7.2 Early Detection	17

2.7.3 Thorough Visual Inspection.....	18
2.7.4 Non-chemical Effective Control of Bed bugs.....	19
2.8 Mechanical Control.....	20
2.8.1 Reducing bed bug establishment	20
2.8.2 Bed bug Trapping	20
2.8.3 Vacuum Cleaning.....	21
2.8.4 Anti-Bed bug Mattress Covers.....	22
2.9 Physical Control.....	23
2.9.1 Steam Treatment	23
2.9.2 Cold Treatment	24
2.10 Chemical Control.....	24
2.11 Resistance of Bed bugs to Bug Sprays	26
CHAPTER THREE	28
3.0 MATERIALS AND METHODS.....	28
3.1 Study Site.....	28
3.2 Study Design.....	29
3.3 Ethical Clearance and Study Area Entry	31
3.4 Descriptive Study on Bed bug Infestations.....	31
3.5 Bed Bug Collection, Laboratory Maintenance and Identification	32
3.5.1 Bed Bug Collection.....	32
3.5.2 Laboratory Maintenance of Bed Bugs	34
3.5.3 Morphological description of identified bed bugs	35
3.6 Determination of Toxicity of Four Insecticides against Bed Bugs, <i>Cimex hemipterus</i>	37
3.6.1 Insecticides Tested.....	37
3.6.2 Insecticide Preparation.....	38
3.7 Topical Assay.....	39
3.8 Data Analysis	40
CHAPTER FOUR.....	42
4.0 RESULTS	42
4.1 Species Composition and Abundance.....	42
4.2 Identification of most commonly used approach to control bed bugs in selected educational institutions	43

4.2.1 Commonly used Method of Bed Bug Control	44
4.2.2 Frequency of Methods/Approaches use.....	45
4.2.3 Products used for Fumigation	46
4.2.4 Duration of Effectiveness of Insecticide Products used for Fumigation against Bed bugs	46
4.2.5 Cost of Controlling Bed Bug in Educational Institutions	47
4.3 Toxicity of Insecticides to Bed Bugs	48
4.3.1 LD ₅₀ of the Four Insecticides used in the Study on <i>Cimex hemipterus</i> F1 adults	48
4.3.2 LT ₅₀ of the Insecticides used in the Study on <i>Cimex hemipterus</i> F1 adults.....	50
CHAPTER FIVE	52
5.0 DISCUSSION	52
CHAPTER SIX.....	59
6.0 CONCLUSION, RECOMMENDATIONS AND LIMITATIONS OF THE STUDY	59
6.1 Conclusion	59
6.2 Recommendations.....	60
6.3 Limitations encountered during the Study	60
REFERENCES	61
APPENDICES.....	75

LIST OF FIGURES

Figure 1: An adult bed bug (*Cimex hemipterus*) 7

Figure 2: Lifecycle of bed bugs (*Cimex hemipterus*) 9

Figure 3: Welts from Bed bug bites 11

Figure 4: Climb-Up Insect Interceptor 21

Figure 5: A map of Ghana showing the study sites in Accra where bed bug samples were collected.
..... 29

Figure 6: Sites and materials from where bed bugs were collected in students’ hall of residence and
dormitories during the study..... 32

Figure 7: Coloured ringed regions showing location of bed bugs in students’ hall of residence and
dormitories during the study..... 33

Figure 8: Bed bug rearing and feeding in the bed bug insectary..... 35

Figure 9: Morphological identification of bed bugs under dissecting microscope. 36

Figure 10: Topical assay..... 40

Figure 11: Bed bug control methods/approaches 44

Figure 12: Frequency of fumigation..... 45

Figure 13: Products used for fumigation..... 46

LIST OF TABLES

Table 1: Examples of Devices for Detecting Bed Bugs	16
Table 2: Characteristics of insecticides used in the experiments	37
Table 3: Species composition and abundance of adult bed bugs collected per study site	42
Table 4: Number and percentage of male and female adult bed bugs used in the study.....	43
Table 5: Cost of controlling bed bugs at the study sites in the educational institutions per year ..	47
Table 6: Log-dose probit mortality data of two populations of bed bugs tested with different classes of insecticides.	48
Table 7: Lethal time to kill 50 % of each population of bed bugs for insecticides used in the study	51

LIST OF APPENDICES

Appendix 1: Management interview guide 75

Appendix 2: Student Questionnaire 77

Appendix 3: A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the Chlorpyrifos insecticide in this study 79

Appendix 4: A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the Lambda-cyhalothrin insecticide in this study 80

Appendix 5: A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the Dursban 4E insecticide in this study 81

Appendix 6: A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the K-Othrine Super insecticide in this study 82

Appendix 7: Percentage of adult bed bugs mortality for both study areas 24 hours post-treatment. 83

Appendix 8: Insecticidal activity of (A) Chlorpyrifos, (B) K-Othrine Super, (C) Lambda-cyhalothrin and (D) Dursban on bed bugs collected from Presec..... 84

Appendix 9: Insecticidal activity of (E) Chlorpyrifos, (F) K-OthrineSuper, (G) Lambda-cyhalothrin and (H) Dursban on bed bugs collected from Mensah Sarbah Hall..... 85

Appendix 10: Mortality over time of one population of bed bugs collected from Presec to 4 commercial products approved for bed bug control..... 86

Appendix 11: Mortality over time of one population of bed bugs collected from Mensah Sarbah hall to 4 commercial products approved for bed bug control..... 87

Appendix 12: T-test 88

LIST OF ABBREVIATIONS

%	Percentage
ANOVA	Analysis of Variance
ARPPIS	African Regional Post – Graduate Programme in Insect Science
CDC	Centers for Disease Control and Prevention
cm	Centimetre
DDT	Dichloro diphenyl trichloroethane
EC	Emulsifiable concentrate
ECBAS	Ethics Committee for Basic and Applied Sciences
EPA	Environmental Protection Agency
g	Gram
g/l	Gram per litre
GHP	General Household Pest
IGRs	Insect growth regulators
IPM	Integrated Pest Management
IRAC	Insecticide Resistance Action Committee
LD ₅₀	Lethal dose at which 50 % of the population are killed
LT ₅₀	Lethal time at which 50 % of the population are killed

MRSA	Multiple-drug Resistant <i>Staphylococcus Aureus</i>
ml	Milliliter
mg	Milligram
PBO	Piperonyl butoxide
PMPs	Pest Management Professionals
Presec	Presbyterian Boys' Senior High School
PTSD	Post-traumatic stress disorder
RH	Relative humidity
SHS	Senior High School
°C	Degree Celsius
μ	Micro
μg	Microgram
UG	University of Ghana
μl	Microlitre

ABSTRACT

The rapid increase in bed bug resistance to pyrethroids call for the development of novel control strategies. Products combining pyrethroids and neonicotinoids have become very popular for bed bug control in Ghana and around the world. However, concerns about evolution of resistance to these compounds have been scantily reported in Ghana. Over the years, various control measures have been employed to control bed bug populations of which chemical insecticides are the most common method used. This study was aimed at identifying commonly used methods/approaches in bed bugs control, determining the lethal dose (LD₅₀) and lethal time (LT₅₀) of some of the frequently used insecticides in some selected educational institutions.

Two bed bug populations were collected from Presbyterian Boys' Senior High School (Presec) and Mensah Sarbah Hall by handpicking and brushing method. Questioner-Interviewer response method was used to identify insecticides and commonly used bed bug control methods in the study. Mass-reared adult bed bugs (*Cimex hemipterus*) (10, 1:1 sex ratio) were treated with the selected insecticides. Each treatment was replicated four times resulting in 40 bed bugs per treatment. Laboratory bioassays were used to measure the toxicity of insecticides after topical application and mortality rate was recorded at 24, 48 and 72 hours post-treatment. Chlorpyrifos (99.5 % purity) and Lambda-cyhalothrin (98.7 % purity) were used as standards for this study.

Fumigation was the most commonly used method in bed bug control for both study sites. Dursban 4E emulsifiable concentrate (480g/L Chlorpyrifos) and K- Othrine Super (Lambda-cyhalothrin 30 g/L and Acetamiprid 30 g/L EC) were observed as the most frequently used products. The highest percentage mortality after 24 hours post-treatment, was recorded to be 100% and 95% for Chlorpyrifos (125 µg/µl). However, K-Othrine Super exhibited a significantly stronger insecticidal activity with LD₅₀ values of 4.35 µg/µl and 4.34 µg/µl against bed bugs collected from Presec and

Mensah Sarbah, respectively. K-Othrine Super was the only insecticide that was uniformly fast-acting and ultimately effective. Lambda-cyhalothrin insecticide showed the least potent insecticidal activity with LD₅₀ values of 23.26 µg/µl and 20.52 µg/µl against bed bugs collected from Presec and Mensah Sarbah, respectively. Results from one-way ANOVA revealed that, there was no significant difference between the insecticidal activities of the two bed bug populations (p=0.473). The shortest LT₅₀ value recorded was 1 hour and 45 minutes for K-Othrine Super against bed bugs collected from Mensah Sarbah whilst the longest LT₅₀ value recorded was 82.16 hours (3 days 10 hours and 9 minutes) for Lambda-cyhalothrin against bed bugs collected from Presec.

This study revealed that population of bed bugs from the two study sites showed different levels of susceptibility to the insecticides.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Study Background

Bed bugs in the tropics are a major biting nuisance. The tropical bed bug, *Cimex hemipterus*, is mainly confined to tropical regions and the common bed bug, *C. lectularius*, is found in temperate climates. The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is an obligate haematophagous insect that has resurged worldwide in the past 15 years (Doggett *et al.*, 2012). Approximately 70 percent of people who are bitten by *Cimex lectularius* have allergic reactions ranging from mild to severe, including itchiness, erythematous rash or urticaria, although fewer people respond to initial exposure (Minocha *et al.*, 2017). For *C. hemipterus*, the percentage of the population that produces a clinical reaction is unknown, with described skin reactions including the formation of papular lesions with associated itch, which resolve around 1 hour after the bite (Rahim *et al.*, 2016).

Before the widespread use of dichloro-diphenyl-trichloroethane (DDT) and other wide-spectrum insecticides in the 1940s and 1950s, bed bugs were part of everyday life (Usinger, 1966). Broad use of these insecticides efficiently controlled infestations and for decades induced a sharp decrease in bed bug populations (Usinger, 1966). Nevertheless, the insecticide resistance of bed bugs to DDT and other compounds was recorded approximately ten years after extensive use (Busvine, 1976). In a research work by (Romero *et al.*, 2009a), the evolution of insecticide resistance to pyrethroids is one of the hypotheses they proposed to explain the sudden resurgence of bed bugs.

Widespread reports of bed bug resistance to pyrethroid insecticides came up after evaluations across the United States of America (Zhu *et al.*, 2010). Pyrethroid resistance in the tropical bed bug, *C. hemipterus*, has been documented in Africa, Sri Lanka and Thailand (Dang *et al.*, 2015).

Studies have verified that some pyrethroid-resistant bed bugs have various resistance mechanisms, including target site insensitivity (*kdr* type), metabolic detoxification enzymes, and resistance to cuticular penetration (Koganemaru *et al.*, 2013). Several strategies for managing pyrethroid resistance in bed bug populations have been suggested. Different studies, such as adding synergistic piperonyl butoxide (PBO) to pyrethroids, were tried to manage pyrethroid-resistant bed bugs (Romero *et al.*, 2009b). Pyrrole chlorfenapyr (Phantom, BASF, Research Triangle Park, N.C.) is increasingly used commercially, although some study reports that this insecticide has a comparatively slow killing action and extremely variable efficacy against bed bugs (Doggett *et al.*, 2012).

Insect growth regulators (IGRs) are potential options for bed bugs management, but they also act slowly and are usually used in conjunction with other fast-acting insecticides by the pest control sector (Romero & Anderson, 2016). Neonicotinoids have become the most commonly used group of insecticides with extensive plant and human protection applications (Starner & Goh, 2012). Several neonicotinoid insecticides coupled with pyrethroids have been launched on the US bed bug control market in recent years (Romero & Anderson, 2016). Intensive use of these combinations by U.S. pest management experts for bed bug control have raised concerns about the rise in pyrethroid resistance as well as the development of neonicotinoid resistance. Some studies show resistance to different neonicotinoids in recently sampled bed bugs with enhanced concentrations of enzymes associated with insecticide detoxification (Romero & Anderson, 2016).

1.2 Justification

Bed bugs present a substantial global public health threat. These species' bites often trigger delayed skin irritations and sometimes lesions due to host allergic immune responses, which could lead to infection. Bed bugs have been found naturally to carry about 40 human pathogens but have not been observed yet to transmit any of them (Cooper & Harlan, 2004). However, there could be mechanical transmission of hepatitis B when infested bed bugs are crushed on abraded skin (Doggett *et al.*, 2012).

Depression, loss of appetite, insomnia are other health implications of bed bugs. Experiences with bed bugs, may also interfere with sleeping pattern and, if severe, may result in later physiological and neurocognitive health effects related with sleep (Doggett *et al.*, 2012). Furthermore, infestation of bed bugs results in social stigma and can trigger financial hardship due to extermination costs and occasional need to replace infested furniture (the latter not as prevalent with the introduction of heat and other non-chemical control methods, but still a factor in some circumstances). The average cost to treat a bed bug infestation in a single-family residence is \geq \$500 (Potter *et al.*, 2010) and to heat treat an apartment costs between \$800-\$1200 (Ashbrook, 2015).

There continues to be frequent reports in the Ghanaian mass media of widespread bed bug infestations of epidemic proportions in the boarding facilities of some second cycle schools in the country (Karikari, 2016). This situation may result in poor sleep and loss of focus among students and may also result in bad academic results. Infestation of bed bugs can result in loss of property such as discarding and/or burning infested products such as mattresses and bed frames (Karikari, 2016). *Cimex* sp. infestations can be a biological indicator of changing social and/or general hygienic conditions and could predict the resurgence of other ecto-parasites such as lice, fleas and

related illnesses (Myles *et al.*, 2003). If the resurgence of bed bugs in shelters and other public facilities is not restricted, there is a danger of uninterrupted and ever-increasing development in bed bug populations resulting in large-scale infestations requiring more common and expensive control attempts (How & Lee, 2010). It is thus prudent to properly determine their susceptibility status to various insecticides on the market as well as the insecticides efficiency and effectiveness as control tools. However, the efficacy, the accessibility and the affordability of bed bug repellents and insecticides in Ghana are not well known. Therefore, determining how effective these insecticides are, could inform the general public on how to cost effectively control bed bug infestations.

1.3 Objectives

1.3.1 Main Objective

To determine insecticide resistance status of bed bugs in selected educational institutions in Greater-Accra Region, Ghana.

1.3.2 Specific Objectives

1. To identify the most commonly used approach to control bed bugs in selected educational institutions in Accra Metropolis.
2. To determine the median lethal dose (LD_{50}) and lethal time (LT_{50}) of insecticide products commonly used in selected educational institutions in Accra Metropolis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origins of Bed bugs

Research by (Panagiotakopulu & Buckland, 1999) record that bed bug parasitism was first exhibited around the Mediterranean region with bats before moving on to humans. Relationships between bed bugs and individuals might not have been that common as hunters and herdsman moved frequently thus not allowing the bed bugs settle. Host and bug cohabitation became easier with the creation of towns and villages, making it easy to establish infestations (Campbell *et al.*, 2016). According to research by (Potter *et al.*, 2011), bed bugs were used to create snakebite antidotes, while Greeks and Romans burned them to loosen the grip of leeches. People back in the days also thought that bed bugs ingested with wine, beans, or eggs could cure many illnesses (Potter *et al.*, 2011).

As civilization and business grew, bed bugs grew and spread across Europe and Asia. The infestation started in Italy by 77 C.E., China by 600 C.E., Germany in the 11th century and France in the 13th centuries (Usinger, 1966). Warmth generated by sleeping and cooking fires made it possible for the bugs to flourish in the palaces of the rich and working-class cottages. However, the poor endured the most based on observation as far back as the 15th century, attributable to lack of careful cleaning (Potter *et al.*, 2011).

2.2 Biology of Bed bugs

Bed bugs are tiny wingless insects that feed on warm-blooded animals' blood alone. Sometimes mistaken for ticks or cockroaches, a mature bed bug is oval, brown to red-brown in colour, wingless and top to bottom flattened (Georgia Department of Public Health, 2007). Bed bugs are nocturnal haematophagous (Karikari, 2016) parasite arthropods belonging to the Family Cimicidae, Order Hemiptera. There are six subfamilies namely: Afrociminae, Cimicinae, Cacodiminae, Haemotosiphonae, Lactrociminae and Primicinae, with 24 genera and 110 species (Karikari, 2016). The adult bed bug (*Cimex lectularius*) is a few millimetres long (about 4 to 7 mm), When a bed bug feeds with blood newly, dull red in colour, its body becomes elongated and swollen. Their eggs are white and slightly curved, about 1 mm long. They are almost colourless or straw-coloured when the bed bugs hatch (Georgia Department of Public Health, 2007).

Young bed bugs generally look smooth, but gradually turn reddish-brown in colour (Chen & Copes, 2013). They have three pairs of legs, a brief, wide head with two prominent antennae, and two dark eyes (Thomas *et al.*, 2004).



Figure 1: An adult bed bug (*Cimex hemipterus*)

When bed bugs are hungry or seek shelter, they migrate through holes in walls, water pipes, heating ducts or canals from one space to another. They move for only a brief range, though, as they have no wings. Because of this, the spread of bed bugs depends mainly on their hosts, the manner in which old furnishings, mattresses and garments are used (Goddard & Deshazo, 2009). It is also stated that bird and bat migration can be a cause of infestation of bed bugs (Romero *et al.*, 2007).

According to a (Chen & Copes, 2013), throughout the day, bed bugs seek refuge in dark, dry locations in beds, mattresses, cracks in walls and floors and furnishings, and may also hide behind images and wallpapers. When hosts are inactive, they feed almost always at night (Heukelbach & Hengge, 2009). They sense and pursue warm-blooded hosts primarily through body temperature perception and carbon dioxide detection (Ter Poorten & Prose, 2005). After emerging from their

hiding locations, bed bugs can migrate up to 20 feet to reach a victim (Kolb *et al.*, 2009). Feeding requires 10 minutes for adult bed bugs and is repeated approximately every three days (Suwannayod *et al.*, 2010). Bed bugs often happen in a unique bite pattern of a three or four linear group, sometimes referred to as a pattern of "breakfast, lunch, dinner" (Romero *et al.*, 2007).

2.2.1 Life Cycle of Bed bugs

Female bed bugs normally lay 1-5 eggs per day. In her 10-12 months lifespan, a female bed bug can lay a total of 200-500 cream-colored eggs in her lifetime (Chen & Copes, 2013). Usually the bed bug eggs are placed in clusters and attached to cracks and crevices or rough surfaces close to adult harbourages with a sticky cement-type substance (Georgia Department of Public Health, 2007). The eggs have a hatching period between 7-10 days. Before maturity, bed bugs go through 5 nymphal phases. They need at least one blood meal at each stage to evolve into the next stadium (Harlan, 2006). Bed bugs must feed on blood for nutrition and growth at all phases. During feeding it takes 10 minutes for complete engorgement to occur and will consume 2-5 times their own body weight in blood during that time.

The host attract bed bugs when they release carbon dioxide from their breath, and various compounds secreted across the skin (Doggett *et al.*, 2012). Factors such as temperature and the availability of food play a major role (Suwannayod *et al.*, 2010).

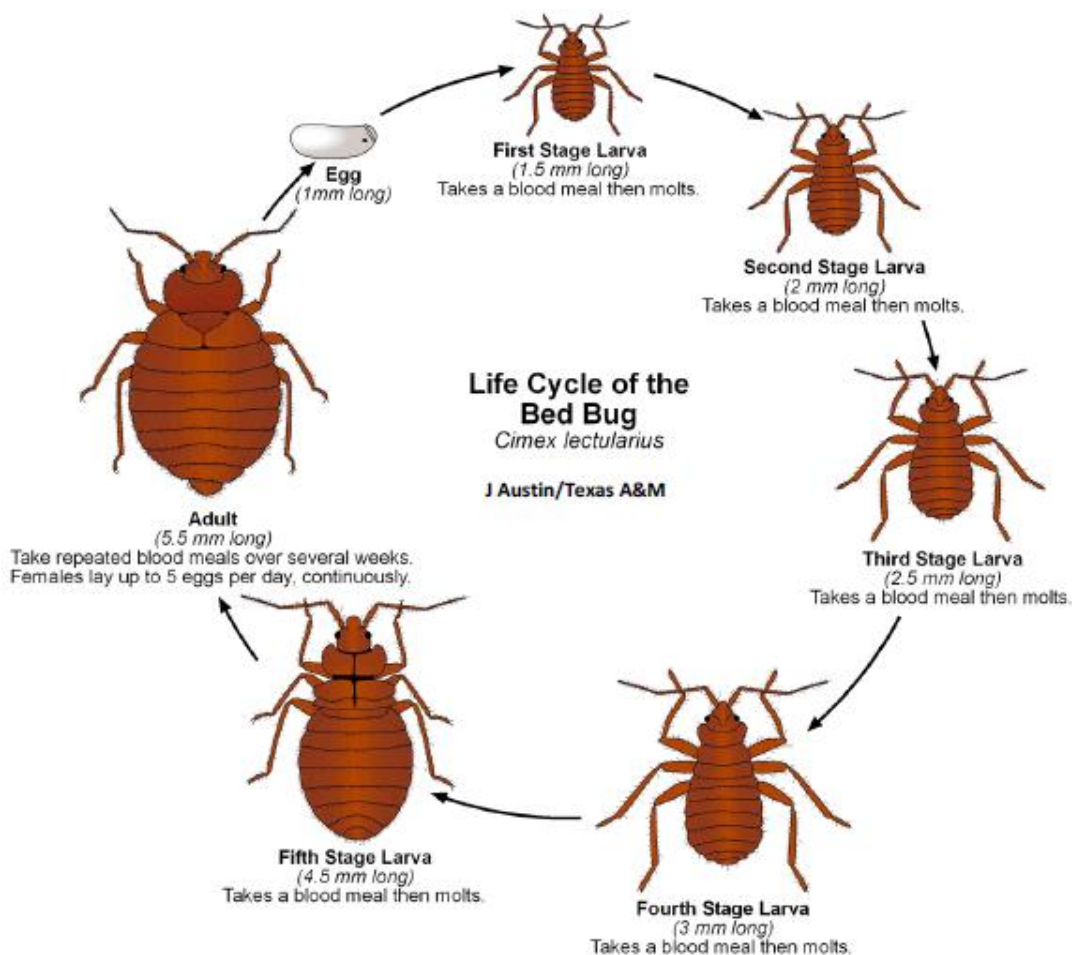


Figure 2: Lifecycle of bed bugs (*Cimex hemipterus*) (Source: Austin, 2015)

It takes approximately 1½ -2 months for a bed bug to go through metamorphosis from an egg hatch through five nymphal stages to adult, with cool temperatures or a blood meal with restricted access extending the growth period. The nymphs have similarities to the small adults with the only difference being small size and presence of a pair of minute wing pads. Female bed bugs tend to be bigger than their male counterparts (Georgia Department of Public Health, 2007).

2.3 Global Distribution of Bed bugs

Per research from numerous authors, bed bugs have been shown to propagate actively and inactively. In other words, they may either be moved by an agent or they migrate on their own (Pereira *et al.*, 2009). Active spreading of bed bugs mostly involves bed bugs migrating from one room to another usually close by. Adult bed bugs have normally been discovered in crevices and cracks in doorways and hallways indicating that the bed bugs have been transported from one place to another (Wang *et al.*, 2011). Passive dispersal occurs when humans facilitate bed bug movement by unconsciously migrating them to new places on or in their clothing, bags, or used furniture (Delaunay *et al.*, 2011). An enhanced interception of bed bugs discovered in traveling bags by domestic quarantine inspectors in Australia is evidence of human facilitated passive dispersal (Davies *et al.*, 2012). Bed bugs used to be more prevalent in the developing world than in the developed world, but owing to enhanced global travel, insecticide resistance and the use of techniques to manage pests that do not influence bed bugs, infestation rates have risen (Dang *et al.*, 2017).

2.4 Health Impacts of Bed bugs

Due to the potential for disease transmission and the physical and emotional distress associated with living with populations of bed bugs it makes this bug to be of significant importance to public health (Eddy & Jones, 2011). According to research (Lai *et al.*, 2016), bed bugs are not known to actively vector disease; however, passive transmission of multi-drug resistant *Staphylococcus aureus* (MRSA) and *Trypanosoma cruzi*, the causative agent of Chagas disease, have been identified through scientific tests in the laboratory. Research by (Potter *et al.*, 2010) indicates that

human reactions to bed bugs bites vary, ranging from no reaction to severe localized reactions and even death; however, death due to anaphylaxis is rare (Goddard & Deshazo, 2009). Infestations of bed bugs can have severe economic and social impacts in the form of extermination costs, decreases in productivity, damage to brand names and lawsuits (Gordon, 2014).

The bite of a bed bug is painless due to the anaesthetic produced during the feeding process. During feeding, a flowing vein or capillary may not always be located by bed bugs on the first probe. This may cause it to probe several times leading to multiple bites (Olson *et al.*, 2013). Typically, the quantity of blood loss due to bed bug feeding does not impact the host adversely. While sleeping, bed bugs feed on any bare skin (faces, necks, shoulders, arms, hands, etc.) The skin aggravations that individuals develop frequently after bite have to do with the saliva injected by the bed bug during feeding.



Figure 3: Welts from Bed bug bites

In any case, a few people don't respond to kissing bug nibbles, while others note a lot of inconvenience frequently with loss of rest from the industrious gnawing. People who are every now and again nibbled by blood suckers may build up an affectability "disorder" that can incorporate apprehension, practically steady disturbance ("anxiety"), and restlessness (Georgia

Department of Public Health, 2007). "Prompt" invulnerable responses may show up from one to 24 hours after a given chomp and can last 1 - 2 days. "Postponed" resistant responses normally first seem one to three (as long as 14) days after a nibble and can last 2 - 5 days. Responses may include the growth of large welts, often > 1 cm, with itching and inflammation (Goddard, 2003). The welts usually die to red spots but can go on for a couple of days. Extreme tingling can keep going for a few hours to days. Rankle like ejections have been accounted for in relationship with numerous blood sucker chomps and hypersensitivity may happen in patients with serious sensitivities. Iron insufficiency in new-born children has been related with extreme pervasions. It was proposed that blood suckers' allergens might be associated with asthmatic reactions (Goddard, 2003). Scratching can trigger infection of the welts. After the expulsion of the stylets from the skin, a few measures of overflowing at the nibble site may happen, this is generally observed on bed sheets as little bits of blood (Steen *et al.*, 2004). Sometimes bed bug feeding can be disrupted by the host's movement (Yao *et al.*, 2014) the bed bug starts feeding again after the victim sleeps (Doggett *et al.*, 2012). Bed bugs cannot be diagnosed by their bites alone. The number of welts is not always the same as the number of bugs that bit the individual. Thus, in one evening, only a few bugs can be responsible for many welts (Klaus Reinhardt & Siva-Jothy, 2007).

According to the Department of Public Health of Georgia (2007), bed bugs were discovered to harbour at least 28 human pathogens and were regarded in the transmission of a broad range of irresistible specialists. Be that as it may, bed bugs have never been demonstrated to organically transmit any human pathogen, including HIV and hepatitis B. In spite of the fact that blood suckers are viewed as all the more an irritation than a wellbeing concern, general wellbeing authorities keep up a degree of enthusiasm because of the likelihood of auxiliary diseases. In any case, the Centers for Disease Control and Prevention (CDC) and the Environmental Protection Agency

(EPA) in the United States of America have issued a joint proclamation saying that "In spite of the fact that bed bugs are not known to transmit illness, they are a vermin of huge general wellbeing significance. This is because:

- Frequent feeding can disturb individuals' rest and make them touchy.
- Seeing nibbles may cause enthusiastic pain in certain individuals.
- Heavy rates of encouraging can bring about huge blood misfortune and inevitably lead to frailty, particularly in malnourished kids.

Bed bugs are commonly associated with sleep deprivation (Doggett *et al.*, 2012). (Potter, 2011) reported that, patients suffered from insomnia due to bites from bed bugs. During the night, patients can wake from the bite of bed bugs because of itching, and scratching can exacerbate the itching feeling, leading to increased sleep disturbance (Thorburn & Riha, 2010). Some patients have troubled sleep by knowing that they had an active or previous infestation in their own bed (Doggett *et al.*, 2012).

2.5 Social Impacts of Bed Bugs

Reports suggest that the effect of bed bug bites are not restricted to physical symptoms alone (Reinhardt *et al.*, 2009). Bed bug bites can usually leave psychological effects on some of the people whose homes gets infested. Often, the psychological problems attributed to living with an infestation of bed bugs can be just as serious as the threat to physical health (Gordon, 2014). Bed bugs can be a menace to the society, it can cause financial instability because infested members in a community may have to dispose furniture, and they would not have initially due to heavy infestation (Karikari, 2016). Surveys have shown that individuals with infestation of bed bugs have

comparable impacts to individuals with post-traumatic stress disorder (PTSD) and delusional parasitosis (Reinhardt *et al.*, 2009). Additionally, the emotional ramifications can be more severe and include delusional parasitosis and thoughts of suicide (Goddard & Deshazo, 2009).

Infestations of bed bugs can have severe economic and social impacts in the form of extermination costs, decreases in productivity, damage to brand names and lawsuits (Gordon, 2014). In a research work by (Wang *et al.*, 2015), it was found that millions of dollars per year are spent trying to control this pest in private residences. Similarly, the hospitality industries spend millions of dollars on control due to the financial impact this pest has on the damage to brand names and product (Gordon, 2014). In addition to costs incurred by the presence and control of this pest, millions of dollars are spent on bed bug litigation (Doggett *et al.*, 2012). Finally, people with bed bugs in their homes often face social rejection and damaged reputations. Given the adverse social, economic and health related issues associated with this pest, many tactics have been developed to control this pest (Gordon, 2014).

2.6 Identification of Bed bug Infestation

Before applying pest-control measures, and to determine which ones would be most appropriate, the presence of bed bugs must first be confirmed. This is done by detecting the insect itself or by observing signs of its presence. Finding a bed bug in one place does not necessarily imply an infestation occurs. However, finding several living bed bugs, hatched and unhatched eggs, nymphs and the brownish skins (exuviae) left behind after moulting, all constitute incontestable proof of an infestation (Quebec, 2018). Detection can be trickier however in cases of low-level infestation.

Indirect signs that bed bugs are present include the following:

1. Dried excrement, appearing as dark spots. Lightly wetted, they turn a rusty or reddish colour, indicating the presence of digested blood (clear proof that a biting insect is present).
2. Blood, appearing as reddish marks, from people scratching bites in their sleep; and also, from crushed bed bugs.

2.6.1 Detection Methods

In determining whether there is a likelihood of a bed bug infestation, there are two methods that are implemented; Basic tools and Active and Passive detection tools.

2.6.1.1 Basic Tools

The most common tools for detecting bed bugs and signs of their presence are: a powerful flashlight, a telescopic inspection mirror, and a magnifying glass or similar device. Since access will be needed to hidden or partially covered recesses, a screwdriver, pliers or crowbar will also be frequently used. Lastly, insects can be collected with insect tweezers and placed in small containers filled with 70 % isopropyl alcohol for later identification.

2.6.1.2 Active and Passive detection systems

In complex cases, such as when only indirect signs are found, or when a dwelling is infested repeatedly, there are devices available to optimize detection. They may be active, attractive or passive, capturing bed bugs as they move around a home (Quebec, 2018).

Table 1: Examples of Devices for Detecting Bed Bugs

Active Systems	Passive Systems
1. Devices generating heat and carbon dioxide (CO₂)	1. Bed bug interceptors for the legs of beds or other furniture
2. Devices generating heat alone	2. Thin interceptors between mattresses
3. Devices combining heat, CO₂ and attractants (pheromones)	3. Sticky interceptors
	4. Pitfall traps

(Source: Quebec, 2018)

2.7 Prevention and Control of Bed Bug Infestation

Management of bed bugs (*C. lectularius* and *C. hemipterus*) infestations often requires many methods including early detection, mechanical removal and the use of heat and steam (Gordon, 2014). Bed bugs are considered one of the most challenging insects to control since they can hide in nests, cracks, empty books and mattresses (Omudu & Kuse, 2010). Early detection of small, localized infestations of bed bugs can be instrumental at effectively eliminating this pest, because locating and managing all members of a small population is easier compared to a large infestation (Gordon *et al.*, 2014). For bed bugs to be detected, pest management professionals (PMPs) use traps and trained dogs. Intercepting traps (often resembling pitfall traps) capture bed bugs as they move to and from the bed or around the floor. The traps allow PMPs to detect bed bug presence and sometimes give information about the source location. In addition to passive traps, active traps use chemical attractants, carbon dioxide and/or heat (Wang *et al.*, 2011).

There are also dogs that have been trained to react to the smell of bed bugs in their presence (Pfiester *et al.*, 2008). Upon the discovery of an infestation, a significant proportion of the

infestation can often be removed through mechanical means. Home owners can eliminate a portion of the population by entombing bed bugs with mattress encasements and creating a permanent barrier between the bugs and the person (Koganemaru & Miller, 2013). Similarly, vacuuming has been proven effective at removing bed bugs. Unfortunately, people living with bed bugs can only mechanically remove bugs that can be found; however, eliminating an infestation requires the control of all members of a population, especially hidden individuals. Heat and steam have been demonstrated to control populations and can frequently kill bugs that were not detected during the initial inspection (Doggett *et al.*, 2012).

2.7.1 Starvation

Due to the need for every organism to feed, starvation has been thought of as one of the means of getting rid of bed bugs. Taking away food is a way of controlling the prevalence of bed bug infestation (Doggett *et al.*, 2012). However, keeping a room vacant to hungry bed bugs may not yield the desired results, as bed bugs usually live long and can go without food for long periods (Usinger, 1966). For example, at 18 ° C, a once-fed bed bug can live for up to 277.1 days (Usinger, 1966), while once-fed bed bugs can survive for about 135 days without a blood meal in a typical hotel room set at a constant temperature of 22 ° C (Doggett *et al.*, 2011). Another reason for its impracticality is that, to use starvation as a tool for controlling bed bugs, one would have to vacate their room for not less than 278 days to achieve desired results according to (Usinger, 1966).

2.7.2 Early detection

For people in accommodations the most impactful way to control bed bug infestation is early detection. Early detection reduces the risk of the infestation spreading. Various methods are employed for early detection of bed bugs (Doggett *et al.*, 2012). This involves the use of trained

canines as practiced in the U.S. A, periodic and careful inspections by housekeepers during routine room maintenance, or through the use of pest supervisors that allow early detection (Doggett *et al.*, 2012). The inspection method should include examining all rooms in hotels, student dormitories, apartment complexes, and other various occupancy buildings. Ideally, risk management should be continuous and enforced even before infestation of bed bugs occurs in adjacent rooms (Doggett *et al.*, 2012). Failure to do so in an apartment complex may cause bed bugs to spread to adjacent units and eventually boost control expenses.

The control of bed bugs is made easier when bugs are detected early (Davio, 2010). Early detection as a control measure of bed bug is a strategy against the growth phase, which involve mainly identifying the presence of bed bugs in its early stages. This can be achieved to promote early reporting by training housekeepers in bed bug detection and training tenants on bed bug recognition. After bed bugs have been detected it becomes easier to limit their spread. Restricting the spread of bed bugs can be accomplished by implementing instant control measures on the identification of bed bugs, quarantining infested spaces and ensuring that infested products are bagged in the room before removal and treatment; before relocation (Doggett *et al.*, 2011) are some of the techniques by which bed bugs can be controlled.

2.7.3 Thorough Visual Inspection

Whenever bed bug invasions are suspected in any place, an exhaustive assessment is required. There will be at least one essential invasion destinations related with areas where individuals rest, sleep or sit for expanded periods (Karikari, 2016). Assessments must be performed seriously, as bed bugs are incredibly level and little (4 – 7 mm, long). A careful investigation is essential to

decide how far reaching the invasion is and the most ideal approach to oversee blood suckers. When directing a review, move gradually and abstain from irritating concealing bugs, so they do not disperse. Remember that in a low invasion, the bed bugs will probably be assumed near resting zones. Things from the resting zone will be at a lower danger of pervasion. Realizing the concealing spots of bed bugs is fundamental to effectively expelling them: they have been found along picture outlines, between the glass and edge itself, and they have been found in the join openings of sleeping pads (Georgia Department of Public Health, 2007).

Assessments must be exhaustive, yet additionally extremely efficient. Parts of furniture should be demolished to the extent that splits and hole can be examined sufficiently to the degree of detail shown lately (Karikari, 2016). In addition to disassembling products, inspection and control involves moving and short movement of furnishings, equipment and various stuff (Karikari, 2016). A deliberate methodology must be taken to guarantee that things in the living region are altogether assessed so as not to investigate every possibility.

2.7.4 Non-chemical Effective Control of Bed bugs

There should be no single management alternative for efficient control of bed bugs. The idea of integrated pest management should be embraced by successful pest supervisors (IPM) (Doggett *et al.*, 2012). This method involves the use of non-chemical controls in combination with the proper use of insecticides (Moore & Miller, 2009). Control of bed bugs should first be performed by identifying the site's pest inspection to determine which regions need treatment. Then, non-chemical control options should be considered which can be followed with the use of insecticide application (Doggett *et al.*, 2012). Finally, assessment of the treatment program's success and risk management processes should be conducted to determine the result of the intervention (Doggett *et*

al., 2012). Control of bed bug infestation can be placed under two broad headings namely non-chemical treatment and chemical treatment.

2.8 Mechanical Control

2.8.1 Reducing bed bug establishment

One method for killing the presence of bugs in your homes or rooms is to step by step decrease their foundations in your homes or rooms. One approach to diminish the foundation of bed bugs is to constrain its pervasion in settlement lodgings by decreasing potential harbourages (Karikari, 2016). This can be accomplished by guaranteeing that breaks and fissure are fixed or constrained in the room. Likewise, furniture and beds ought to be developed with materials, for example, smooth metals and plastics as opposed to wood. What is more, sleeping pad ought to be encased (Doggett *et al.*, 2011). This guarantees fewer concealing territories are accessible for blood suckers. Some bedding encasements are nibble confirmation, and these encased invaded sleeping pad avoid the break of bed bugs in this manner keeping beddings from being disposed of (Pinto *et al.*, 2007). The shade of encasement should likewise be mulled over as white encasement have the extra advantage of making bed bug recognition simpler.

2.8.2 Bed bug trapping

Bed bug catching is one of the best methods for controlling the quantity of bugs in a given spot (Anderson *et al.*, 2009). One type of trap that has been utilized for quite a while is the Pit-Fall trap (Wang *et al.*, 2009). It has been utilized in research programs for the checking of populaces (Cooper *et al.*, 2015). Some portion of the Pit Fall trap is the gadget known as the Climb-Up Insect Interceptor, which goes about as both an obstruction and a screen for bed bugs. This gadget

comprises of an ultra - smooth plastic bowl with an external gadget like a bowl (Doggett *et al.*, 2012). It has been demonstrated to be more successful at identifying bed bugs than visual investigation. Although significant time and effort are sparing, non-chemical means do not provide lingering control, and there is a risk of re-invasion whenever treated things are returned to the pervaded zone.



Figure 4: Climb-Up Insect Interceptor

2.8.3 Vacuum Cleaning

Another method of reducing bed bugs in homes is vacuum cleaning. This can reduce the biomass of the bed bug in an infestation rapidly and even remove many eggs (Doggett & Russell, 2009). Vacuum machines are affordable, readily available, require little or no teaching to operate and do not require any operator licensing. Vacuum cleaning presents a slight risk of spreading an infestation (Doggett *et al.*, 2012). All surfaces where traces of bed bugs are found should be

vacuumed thoroughly, using the appropriate accessory (brush, crevice nozzle, etc.). Pest control specialists use a machine equipped with an absolute high-efficiency (HEPA) filter. Highly effective in pest control, it is designed to capture particles of extremely small size, including fibres, species spores, dust mites, pollen grains and other allergens (Quebec, 2018). Though essential to preparing the infested area, the vacuum cleaner alone will not eliminate all bed bugs, since they are often hiding in inaccessible places, nor will it eliminate the eggs, which are covered with a sticky substance that resists even powerful suction (Quebec, 2018).

2.8.4 Anti-Bed bug Mattress covers

Anti-bed bug covers for mattresses and box springs create a physical barrier that prevents bed bugs from spreading to other areas and are trapped inside the cover where they will eventually die of hunger. Mattresses that have developed holes already but not infested can be covered to prevent bed bugs from sheltering in the intricate habitat provided by the bed (Doggett *et al.*, 2012). Anti-bed bug mattress covers can be used to prevent infestation from a used mattress or box spring, or can be installed after an intervention to prevent discarding an infested mattress. Using mattress covers will also facilitate later inspection, since they render a customary habitat inaccessible. Bed bugs can survive a long time without feeding, so mattress covers should be left on permanently to be effective. If damaged, they should be replaced immediately. Many people in the pest control sector have viewed the use of mattress covers as a technique for further reducing pesticide hazards to human exposure and avoiding liability for insecticide application on the mattress (Olson *et al.*, 2013).

2.9 Physical Control

Physical control strategy utilized as a method for controlling bed bug invasions have demonstrated their adequacy. They will frequently control a pervasion all the more rapidly, while constraining pesticide use and the related dangers of presentation (Quebec, 2018).

2.9.1 Steam Treatment

Steam treatment is profoundly powerful, since it can kill bed bugs in each period of the existence cycle (from egg to adult). The introduction of all phases of *C. lectularius* to temperature of 45 °C for one hour will kill the creepy crawly (Johnson, 1941) and at temperatures more than 60 °C, all blood suckers are quickly killed (Boase & Naylor, 2014). Steam treatment can be connected to each potential concealing spot of blood suckers: places where eggs, fairies and grown-ups have been found, and any place invasion is suspected. The spout ought to be held at a specific good way from the surface being dealt with, and moved gradually, enabling 10 to 15 seconds for each 30 cm of movement. Warmth can be connected by means of the utilization of steam, through the washing of invaded apparel and bedding, by means of hot washing and drying (Naylor *et al.*, 2008), and using contained or circled heat medicines (Paul & Bates, 2000). Steam must not be connected to fragile things, for example, cow hide, acrylic, vinyl or material, painted surfaces, wood completes, covered wood, re-enacted wood trim, plastic, backdrop or other stuck sur-faces, nor electronic machines. The warming of entire rooms accompanies the danger of spreading the invasion, as bed bugs will look for cool spots which has a temperature over 30 °C to 35 °C (Doggett *et al.*, 2012).

2.9.2 Cold Treatment

This kind of treatment requires freezing temperatures to dispose of bed bugs in each period of the existence cycle (from egg to adult). Cold treatment can be connected to most surfaces, including toys, plastics, books, electrical apparatuses, garments and materials. Items being treated must be left in the freezer at a temperature of -18 °C or colder for at least 84 hours (3.5 days). Avoid compacting them, and leave sufficient space between items for the prescribed internal temperature to be reached quickly. Under temperatures of – 17 °C for two (2) hours, 100 % of all life stages of *C. lectularius* were killed (Naylor & Boase, 2010). There are different frameworks that utilize gases to kill bed bugs; in any case, these can work just under high weight. The inconvenience being that little air flows can scatter bed bugs (Feldlauer *et al.*, 2014).

2.10 Chemical Control

Insecticides alone are likely insufficient to control bed bug infestations. Their use must be combined with a program of removing and cleaning infested beds, bedding, and other harbourage sites then following up with a regular detection program to ensure treatment was effective (Georgia Department of Public Health, 2007). The most effective bed bug pesticides are available for commercial purchase. Professionals also have the equipment and expertise that allow a more effective application of insecticides than residents could do themselves. They additionally have the training to detect and isolate infestations, which often allows for more effective control. Insecticides may be applied as liquid insecticide sprays, dusts, or aerosols. Insecticide sprays (including insect growth regulators) are used to spot treat bed bug harborages in cracks and crevices, behind baseboards, along ceiling-wall and floor-wall junctions, in closets,

behind crown molding, and along window and door frames (Georgia Department of Public Health, 2007).

Dusts have the advantage over liquid insecticides in that bed bugs walking on dusted surfaces will become covered in the dust resulting in superior kill (Kells, 2010). Insecticidal dusts are used to treat wall voids and electrical outlets. It is not advisable to use the insecticidal dust outside as it will likely be carried away by air/wind currents. Desiccant dusts kill bed bugs by absorbing the protective wax layer on the outside of their body. Aerosol formulations are used in cracks and crevices, the under sides of drawers, drawer slides, nail holes, chipped paint, carpet tack strips, and in the wood framing of the box springs (Kells, 2010). Some aerosol products (not all) can be used on upholstered furniture. Many aerosol products have the advantage of being able to kill bed bug eggs. Most insecticide sprays and dusts will not.

Liquid insecticide formulations include:

- Products containing the botanical insecticide pyrethrin, which gives quick knockdown but little long-term control.
- Various synthetic pyrethroid products (cyhalothrin, bifenthrin, deltamethrin, and permethrin)
- Newer types of products including the pyrrole insecticide chlorfenapyr (Phantom) and the insect growth regulator hydroprene (Gentrol), which cause sterility in adults (Georgia Department of Public Health, 2007).

However, both newer products take up to several days to be effective. Insecticides applied as dusts cling to the pest's cuticle, wearing away the insect's protective wax covering or poisoning the insect when it grooms itself. Several dust products used in bed bug management include boric acid, diatomaceous earth, fumed silica, and formulations of pyrethroids. These materials can provide

long-term control as part of an integrated program if they are placed in out-of-the-way places—such as under baseboards or in wall voids—that don't get wet (Kells, 2010). The first application may not give complete or immediate control, and additional treatments may be necessary. In most cases, bed bug infestations can be resolved in three to four visits. In most situations, the bed bug populations should be dramatically reduced following the initial visit. The appearance of new evidence of bed bugs after a series of service visits does not necessarily indicate a service failure; the new bed bugs might be reintroductions from other infested locations. Regardless of how severe the infestation or how complex the environment, there is one way that bed bug infestations can be eliminated with absolute certainty. Structural fumigations, while an extreme and costly method, will effectively eliminate bed bugs from an infested environment (Georgia Department of Public Health, 2007).

2.11 Resistance of Bed bugs to bug sprays

The Insecticide Resistance Action Committee (IRAC) defines insecticide resistance as a reduction in the sensitivity of an insect population to an insecticide (Palenchar *et al.*, 2015). Research studies from Wang and Wen (2011) discovered that despite guidelines on how to apply insecticides being followed adherently, it repeatedly failed to achieve the desired level of control. It is important to note that many of insecticide products are formulated using a specific class of insecticides that has low toxicity to mammals but high toxicity to insects (Kilpinen *et al.*, 2011). Any chemical that kills applies selection pressure, which together with genetic heterogeneity within the population, results in evolution that may translate into the loss of effectiveness of the insecticide (Gordon, 2014). Molecular mechanisms of insecticide resistance fall within three categories: reduced cuticular penetration, target site insensitivity and enhanced metabolism (Mamidala *et al.*, 2011).

Pyrethroids cause the nerves to fire continuously until the insect loses control of its bodily functions and dies (Wang and Wen, 2011). Many of the liquid spray products, aerosols, and dusts contain pyrethroid insecticides (Wang and Wen, 2011). Unfortunately, pyrethroid insecticides have been used so much throughout the world that many bed bug populations have developed resistance to them. Resistance means that the bed bugs have developed the ability to survive the pyrethroid exposure (Miller, 2015). Resistant bed bugs are also able to pass the resistance in the form of gene transfer to their offspring. The development of resistance has added to the current bed bug population explosion (Wang and Wen, 2011). In 2011, resistance to organophosphates was reported for both *C. lectularius* and *C. hemipterus* bed bugs from Thailand (Tawatsin *et al.*, 2011), and for *C. lectularius* bed bugs collected from Denmark (Kilpinen *et al.*, 2011).

CHAPTER THREE

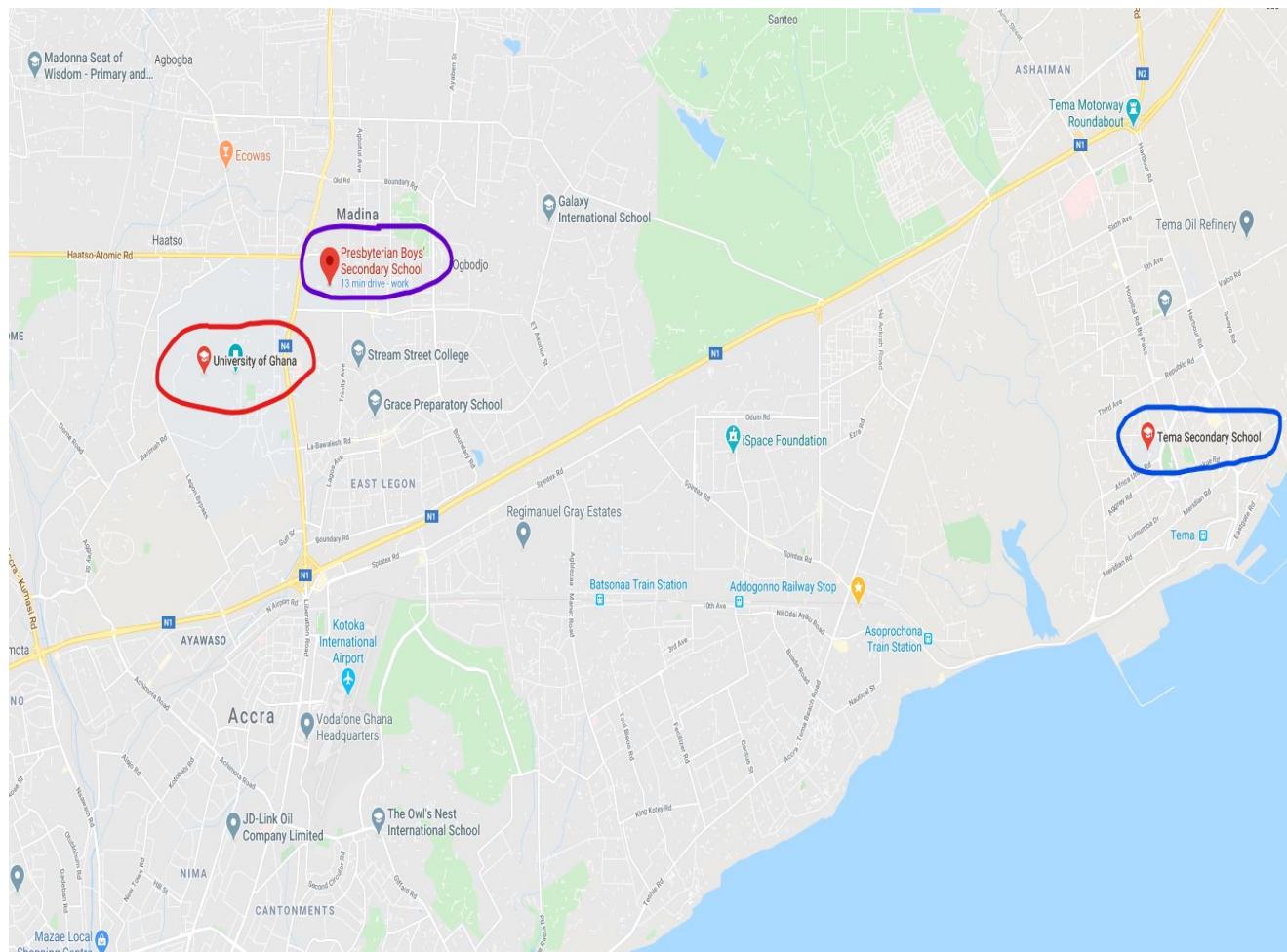
3.0 MATERIALS AND METHODS

3.1 Study site

The study areas were the University of Ghana (Latitude 5° 39' 1.79'' N and Longitude 0° 11' 7.80'' E), Presbyterian Senior High School (Latitude 5° 39' 28.79'' N and Longitude 0° 10' 16.80'' E) and Tema Senior High School (Latitude 5.6537° N and Longitude 0.0257° E) (Figure 5) in the Greater Accra Region of Ghana. Within these educational institutions, bed bugs were sampled from rooms and dormitories.

Greater Accra's vegetation cover is connected with landscaping, parks, urban agricultural parcels, regeneration after clearing, some remaining forests and wetland vegetation around lagoons (Stow *et al.*, 2013). Located in the dry equatorial climate area, the Greater Accra Region experiences two rainy seasons. The major rainy season begins in the month of March, peaks in May or June and ends in mid-July, while the minor rainy season begins in mid-August and ends in October. The annual total rainfall is about 700 to 800 mm. The average temperature condition varies from about 26 °C in August to 30 °C between March and April, and the relative humidity in most part of the Region falls between 65 % and 75 % during the day especially in the afternoons (Achonduh *et al.*, 2008).

The collection of bed bugs was conducted during the minor rainy season, from August to October 2018. The study sites were chosen as the sampling sites based on reports from previous studies, which discovered that bed bug outbreaks have been consistently high in these educational institutions compared to other educational facilities in the municipality.



- University of Ghana
- Presbyterian Boys' Secondary School
- Tema Senior High School

Figure 5: A map of Ghana showing the study sites in Accra where bed bug samples were collected.

3.2 Study design

A multi-method evaluation study design was used to acquire information on the presence or absence of bed bugs. The methods included questionnaire administration, direct observations, quantitative data collection and statistical test assessment. Questionnaires were used to assess the presence of bed bugs status in the educational institutions, the control methods/approaches used in

bed bug control, the products used for fumigation. Permission was also sort to carry out collection. Observations were made during the sample collection and compared with existing data in other research studies.

Convenient sampling method was used to select one tertiary institution (University of Ghana, Legon) and two Senior High Schools (Presbyterian Boys' Senior High School and Tema Senior High School) based on reports from previous studies of their high consistency of bed bug outbreaks. Stratified random sampling and convenient sampling methods were then used to select the halls and houses in the various institutions. Stratified random sampling is a probability sampling method where the entire target population is divided into distinct subgroups called strata, and then subjects randomly chosen proportionally from each stratum (Kemper *et al.*, 2003). Samples were taken at 5 University of Ghana halls of residence, one (1) house at Presbyterian Boys' Senior High School and two (2) houses (one male and one female) at Tema Senior High School. The strata on University of Ghana campus were mixed halls on main campus, all-male halls, all-female halls, private hostels and the diaspora. The halls randomly selected from the strata comprised the two single sex halls, that is Commonwealth hall (all-male) and Volta hall (all-female), Jean Nelson AKA Hall from the diaspora, Evandy hostel form Private hostels and from the mixed halls on main campus strata, convenient sampling was used to select Mensah Sarbah Annex A, B, C and D due to fumigation of the main hall of Mensah Sarbah and other halls in this category at the period of bed bug sampling. One house (House 10) at Presbyterian Boys' School was also selected due to fumigation activities during the sampling period. The strata for Tema Senior High School was gender and two houses randomly selected.

Samples were collected from 5% of rooms in the halls and houses selected. With the single sex halls and houses, the strata were the blocks and rooms were selected at random from each block

in equal proportions to make up 5% of the rooms in the halls and houses. With the mixed halls and mixed school (Tema Senior High School), they were stratified into gender and then further stratified into blocks; that is, 2.5% of rooms were randomly selected from female blocks and same was done with male blocks.

3.3 Ethical clearance and study area entry

Ethical clearance was sought to conduct this study and the proposal approved by University of Ghana Ethics Committee for Basics and Applied Sciences with reference number: ECBAS 006/18-19. Introductory letters from the African Regional Postgraduate Programme in Insect Science (ARPPIS) were sent to the Heads of the educational institutions to seek permission for sample collection.

3.4 Descriptive study on bed bug infestations

Preceding the collection of bed bugs in the field, questionnaires were administered by random sampling method to assess people's knowledge on bed bugs as well as the control methods/approaches used against bed bugs and also the products used. This was administered by the Questioner-Interviewer Response Method. Respondents willing to participate in the questionnaire answering process included five (5) out of seven members of management (Appendix 1) and 150 students of the study areas (Appendix 2).

3.5 Bed bug collection, laboratory maintenance and identification

3.5.1 Bed bug collection

Bed bug populations used in this study were collected by physical handpicking and brushing methods with the aid of a flashlight and a pair of forceps. Bed bugs were collected from metal bed frames and in cracks or tiny perforations on walls and were quickly transferred into rearing cages (Figure 6). Signs of bed bug infestation were identified by the presence of eggs, exuviae and crushed bed bug with blood stains on bed sheets on surfaces around harbourage (Figure 7). The rearing cages were made up of about 300 ml transparent plastic containers, with a height of 10 cm and a diameter of 6.5 cm. A fine cotton mesh was cut to size and used as a cover with the aid of a rubber band forming a tight seal.



Figure 6: Sites and materials from where bed bugs were collected in Students' hall of residence and dormitories during the study. A: Collection of bed bugs from a metal bedframe using flash light and forceps; B: Bed bugs in a wall hidden in electrical socket.

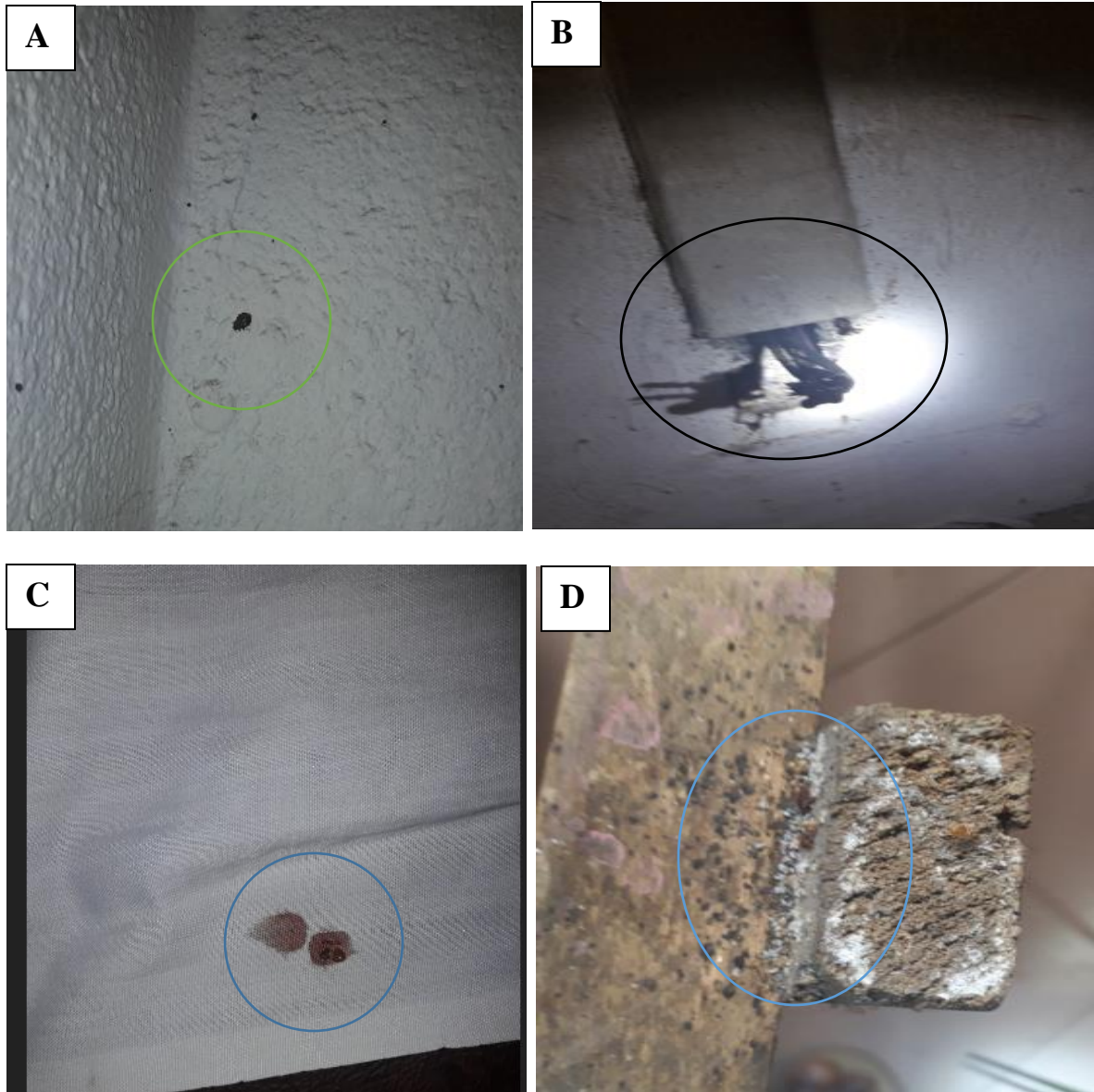


Figure 7: Coloured ringed regions showing location of bed bugs in students' hall of residence and dormitories during the study. A: Adult bed bug on a wall; B: A hole in an exposed electrical wiring harbouring bed bugs. C: Crushed bed bug with blood stain on a bed sheet. D: Eggs and exuviae of bed bugs on a wooden bed.

3.5.2 Laboratory maintenance of bed bugs

Twenty (20) bed bugs were held in each container that included a harbourage constructed from a cardboard rectangular-cut (140 by 100 mm) where the bugs can clump and conceal themselves (Figure 8 A). The fine cotton mesh allows all stages of the bugs to feed on blood source. It also prevents bugs from escaping during removal of bugs for testing. The bugs were kept at 26 ± 2 °C, 65 ± 10 % RH, and a photoperiod of 14:10 (L: D) h. The colony of bed bugs in each container was inverted on a blood source of a restrained rabbit for feeding (Stutt & Siva-Jothy, 2001) once every three days for 10 minutes per each container (Figure 8 B). Eggs that had been laid were transferred into a new container and after 10 - 14 days of hatching the first instars were transferred into a new container. The bed bugs were reared according to their developmental stages, first nymphal instar to the adult stages. It took 2.5 - 3 months for a complete cycle from egg to mated adult bed bug.

Mesh covers were intermittently changed, since bed bug excreta can block the holes in the mesh, thereby reducing the surface area through which the bugs can feed. Occasionally, exuviae were also removed from the bottom of the container using lengthy forceps as the exuviae can create an unnecessary physical barrier between bed bugs and blood meal. Heavily soiled harbourages are also periodically replaced and a soft bristle brush used to dislodge most adults or larger nymphs that still cling to the old harbourage. Old containers, forceps, brush and old harbourages were soaked in 70 % alcohol over night to kill any living bugs that did not transfer. Assessment of insecticides started 8 – 10 days after adult emerged, and were fed two days prior to the experiment.



Figure 8: Bed bug rearing and feeding in the Bed bug Insectary. A: Bed bugs in rearing containers on shelves; B: Blood feeding of bed bugs using a restrained rabbit.

3.5.3 Morphological description of identified bed bugs

Morphological identification of adult bed bugs was done using taxonomic keys outlined by (Usinger, 1966). Body parts of key importance such as pronotum, hair on pronotum, shape of abdomen and antennae were body parts selected for the identification because they are features that have previously been used to distinguish one bed bug species from another (Usinger, 1966).

Cimex hemipterus also known as the Tropical bed bug identified in the laboratory had a wider pronotum as compared to other species of bed bugs. The lateral flanks of the pronotum were not upturned like that of *Cimex lectularius* (Figure 9 A) hence making the neck of the insect look long since most of it is not covered by the pronotum. *Cimex hemipterus* also had a narrower longer abdomen compared to the other species of bed bugs (Figure 9 B). Moreover, coxae of *Cimex*

hemipterus had a zigzag shape at the proximal end which attaches to the insect's body. In addition, the length to width ratio of the femora is 2.9 – 3.5 according to (Usinger, 1966).

On the other hand, most of the neck of *Cimex lectularius* was dorsally invisible since it was covered by the pronotum and the abdomen was comparatively wider and oblique and gives the insect a shorter look (Figure 9 A). In this bed bug species, the coxae had a hook-like shape at the proximal end, which attaches to the insect. The length to width ratio of the femora is 3.4 – 4.1 according to (Usinger, 1966).

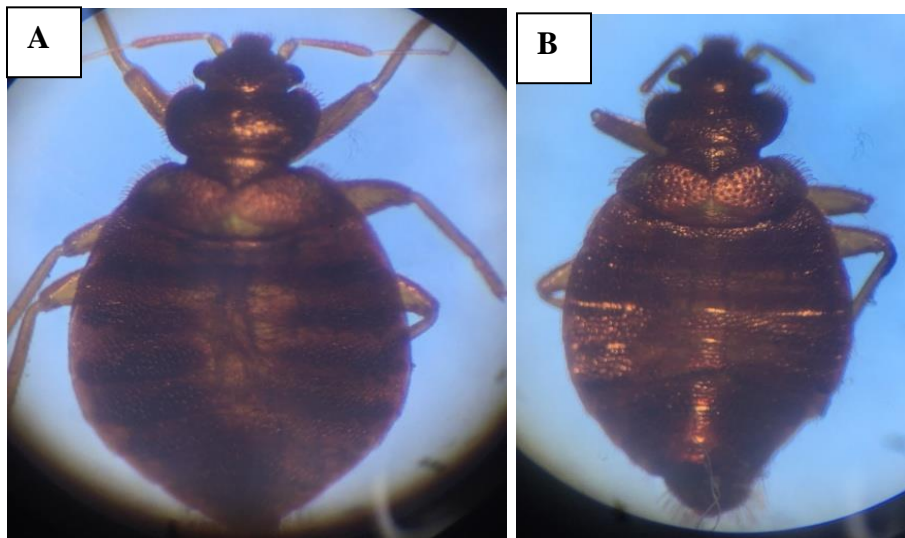


Figure 9: Morphological identification of bed bugs under dissecting microscope A: *Cimex lectularius* B: *Cimex hemipterus*.

3.6 Determination of toxicity of four insecticides (belonging to 3 classes of insecticide) against bed bugs, *Cimex hemipterus*

3.6.1 Insecticides Tested

Dursban 4E emulsifiable concentrate containing 480g/L Chlorpyrifos and K-Othrine Super containing two active ingredients (Lambda-cyhalothrin 30 g/L and Acetamiprid 30 g/L EC) were recorded as the most frequently used product for fumigation by management of the selected educational institutions. Chlorpyrifos (99.5 % purity) and Lambda-cyhalothrin (98.7 % purity) were used as standards for this study. Table 2 gives the characteristics of the insecticides used in the toxicity bioassays.

Table 2: Characteristics of insecticides used in the experiments

Name of insecticide	Class of insecticide	Formulation and content
Chlorpyrifos	Organophosphate	Dust, 99.5 % purity
Lambda-cyhalothrin	Pyrethroid	Dust, 98.7 % purity
Dursban 4E	Organophosphate	Emulsifiable concentrate (EC) 480 g/L Chlorpyrifos
K-Othrine Super	Pyrethroid / Neonicotinoid	Lambda-cyhalothrin - 30 g/L and Acetamiprid - 30 g/L EC

3.6.2 Insecticide preparation

Stock solution and serial dilutions were prepared for all the insecticides at different concentrations as shown in (Appendix 3, 4, 5 and 6). In all cases hexane was used as solvent to dissolve the active ingredients and then the appropriate volumes were transferred into vials and then evaporated to dryness under nitrogen gas. The residue was then redissolved in acetone to obtain the appropriate dose for application to the bed bugs.

Stock solution of Chlorpyrifos (10 mg/ml) was prepared by dissolving 10 mg in 1 ml hexane from which 1 mg/ml solution was obtained. The following concentrations were then prepared by serial dilutions to give 125 $\mu\text{g}/\mu\text{l}$, 25 $\mu\text{g}/\mu\text{l}$, 5 $\mu\text{g}/\mu\text{l}$ and 1 $\mu\text{g}/\mu\text{l}$ (Appendix 3).

Similarly, stock solution of Lambda-cyhalothrin (10 mg/ml) was prepared by dissolving 20 mg in 2 ml hexane from which 1 mg/ml solution was obtained, then 5-fold serial dilutions were prepared to give 125 $\mu\text{g}/\mu\text{l}$, 25 $\mu\text{g}/\mu\text{l}$, 5 $\mu\text{g}/\mu\text{l}$ and 1 $\mu\text{g}/\mu\text{l}$ (Appendix 4).

Stock solution of Dursban 4E (10 mg/ml) was prepared by dissolving 0.25 ml in 1.2 ml hexane from which 1 mg/ml solution was obtained, then 4- and 5-fold serial dilutions were prepared to give 25 $\mu\text{g}/\mu\text{l}$, 6.25 $\mu\text{g}/\mu\text{l}$, 1.5 $\mu\text{g}/\mu\text{l}$ and 0.3 $\mu\text{g}/\mu\text{l}$ (Appendix 5).

Similarly, stock solution of K-Othrine Super (10 mg/ml) was prepared by dissolving 0.33 ml in 1 ml hexane from which 1 mg/ml solution was obtained. The following concentrations were then prepared by serial dilutions to give 10 $\mu\text{g}/\mu\text{l}$, 5 $\mu\text{g}/\mu\text{l}$ and 0.5 $\mu\text{g}/\mu\text{l}$. From 1 mg/ml solution a 0.1 mg/ml solution was obtained, then 10-fold serial dilutions were prepared to give additional concentrations 0.05 $\mu\text{g}/\mu\text{l}$ and 0.005 $\mu\text{g}/\mu\text{l}$ (Appendix 6).

3.7 Topical Assay

Mass-reared bed bugs (*Cimex hemipterus*) F1 comprising of adults were used in this experiment. Evaluation of insecticides were carried out 8 – 10 days after adult emergence. Forceps was used to remove adult bed bugs from a colony and placed in a non-sticky surface of 90 mm petri dishes (manufactured by Bioline) and chilled for 1 minute at a temperature of -20 °C in a refrigerator in order to slow their movement because their fast movement could lead to the bugs escaping.

Slowing the movement of bugs by cooling excludes the need to use carbon dioxide, which in experiential research is known to have a negative effect on insect behaviour (Feldlaufer *et al.*, 2014). Ten (10) bed bugs (1:1 sex ratio) were used for each replicate of each insecticide. Each treatment was replicated four times resulting in 40 bed bugs per treatment.

Eppendorf pipette equipped with a 10 µl Neptune pipette tip was used to administer 1 µl of the dose of the insecticides topically to the dorsal surface of the abdomen and control bugs received 1 µl of acetone only (Figure 10). The treated bugs were then transferred into 300 ml transparent plastic containers, with a height of 10 cm and a diameter of 6.5 cm and covered with a fine cotton mesh cut to size with the aid of a rubber band to form a tight seal. Temperature and humidity in the laboratory were kept at 26 ± 2 °C and 65 ± 10 % respectively. Mortality rate was recorded at 24, 48 and 72 hours by gently probing each bed bug with a forceps and bed bug was considered dead if it was not moving or could not turn upside down.

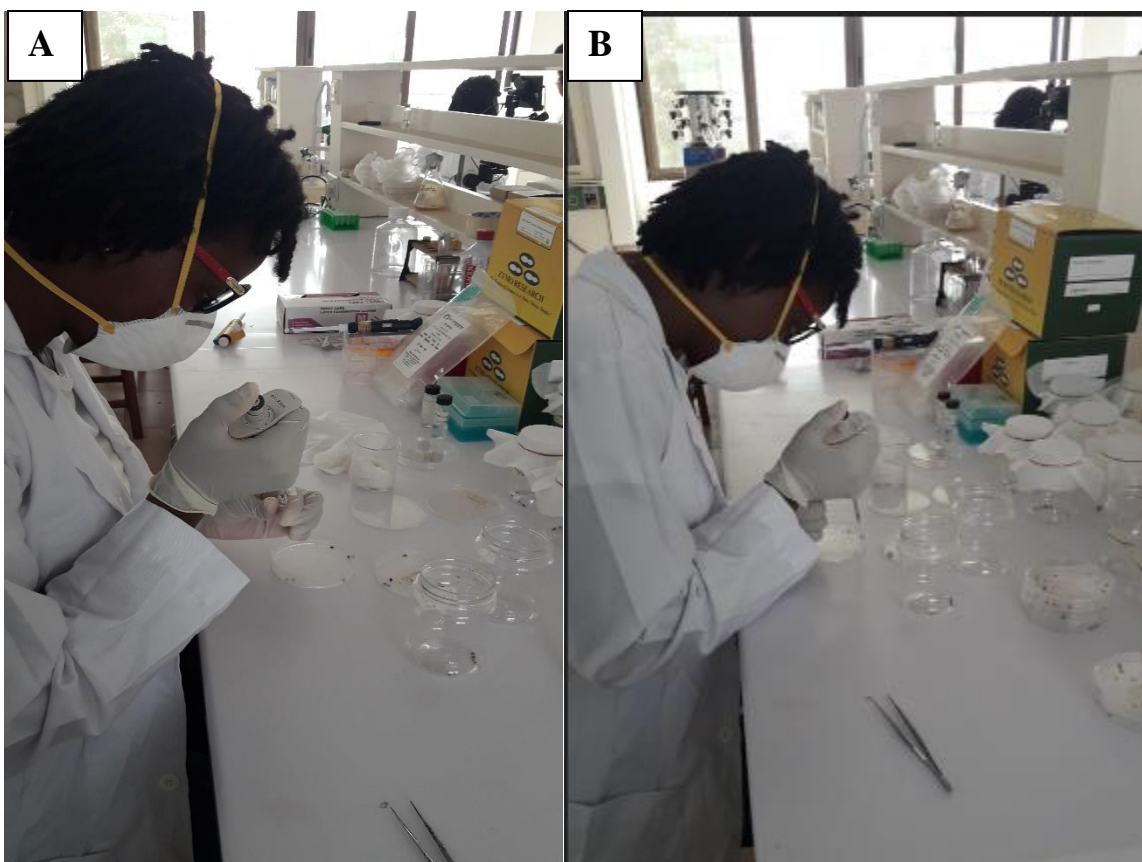


Figure 10: Topical assay. A: Picking of insecticide solution from 1.5 ml Eppendorf tube with an Eppendorf pipette equipped with a 10 μ l Neptune pipette tip; B: Topical application of insecticide to the dorsal surface of the abdomen of bed bugs.

3.8 Data Analysis

The charts for method/control approaches and products used in the study areas from the questionnaires administration was analyzed using Microsoft Word 2013. All data collected for the study were entered into the Microsoft Excel Spreadsheet and imported into SPSS version 16.0 and probit analysis used to determine lethal dose at 50 % mortality (LD_{50}).

Significant differences between the two populations exposed to Dursban 4E, K-Othrine Super, Chlorpyrifos and Lambda-cyhalothrin were determined by ANOVA and p -values ≤ 0.05 were used

to indicate level of significance (SPSS version 16.0). Significant differences between LD₅₀ values of both populations of bed bugs exposed to each insecticide were determined by the failure of the confidence intervals (CI) to overlap. LD₅₀ and LT₅₀ values were calculated by means of SPSS version 16.0 probit analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Species composition and abundance.

Out of 784 adults bed bugs collected from the study sites for rearing in the bed bug insectary, 602 (76.8 %) were morphologically identified as *C. hemipterus* and 182 (23.2 %) as *C. lectularius* (Table 3).

Table 3: Species composition and abundance of adult bed bugs collected per study site

Species	Mensah Sarbah	Common-wealth	Volta hall	Evandy hostel	Jean Nelson	Presec	Temasco	Total	% Quantity
<i>Cimex hemipterus</i>	235	40	5	0	7	250	65	602	76.8
<i>Cimex lectularius</i>	5	131	5	0	11	10	20	182	23.2
Total	240	171	10	0	18	260	85	784	100

Cimex hemipterus was the predominantly collected bed bug samples from most study sites. However, due to the death of all samples from Temasco and the low numbers of bed bugs obtained from Commonwealth, Volta and Jean Nelson Halls, colonies could only be successfully raised from Mensah Sarbah Hall and Presec samples, which were used for the bioassay experiment of this study. Though fewer *C. lectularius* were comparatively collected, their numbers were higher at Commonwealth Hall than any of the other study sites (Table 3). From the collected sample 452 (57.7 %) were males and 332 (42.3 %) were females (Table 4). All collected bed bugs from Mensah

Sarbah Hall and Presec were used in establishing the colony subsequently used in the experimentations.

Table 4: Number and percentage of male and female adult bed bugs used in the study

Sex	Number Obtained	% Number obtained
Male	452	57.7
Female	332	42.3
Total	784	100

4.2 Identification of most commonly used approach to control bed bugs in selected educational institutions

In this study, high incidence of bed bug infestation was recorded in the dormitories and halls of residence of the study sites. From the questionnaire administered to the management of the residencies (dormitories and halls), 3 respondents representing 60 % of the total respondents stated that they have known about the infestation for more than 3 years, and the remaining 2 respondents representing 40 % stated that they have known about the infestation for 1-3 years. Student respondents to the questionnaire on how long they have known about the infestation responded as follows: 80 respondents representing 53 % stated between 0 - 6 months, 38 respondents representing 25 % stated between 6 - 12 months, 23 respondents representing 14.7 % stated between 1 - 3 years and 9 (8.3 %) respondents stated more than 3 years.

4.2.1 Commonly used method of bed bug control

From the management questionnaire on the question: “What control methods/approaches do you use to control bed bug infestation?” Four of the management respondents representing 80 % of the total respondents stated that they use fumigation as a method of dispensing insecticides to get rid of the bed bugs. One respondent representing 20 % stated that the Gas/Heat method was adopted (Figure 11).

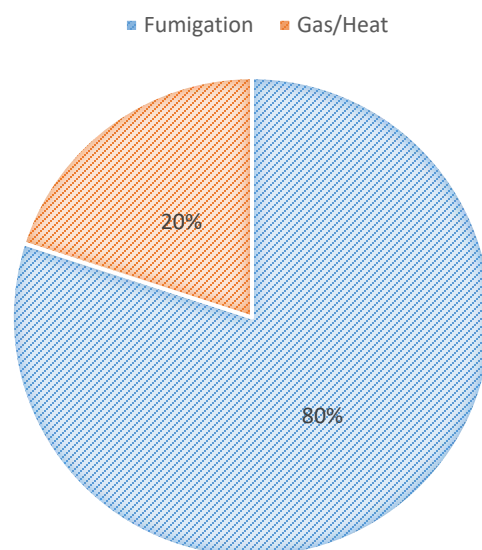


Figure 11: Bed bug control methods/approaches used by management in the study sites.

Generally, all the respondents in the survey indicated the use of acceptable control strategies such as insecticide fumigation practices and use of heat method in bed bug management. One hundred and twenty-one respondents, representing 80.6 % of students, stated that the use of fumigation method was effective while, 29 representing 19.4 % stated that fumigation was not effective against the control of bed bugs infestation in the halls/hostels and dormitories.

4.2.2 Frequency of methods/approaches use

According to the willing participant from the management of the educational institutions, fumigation is the common method used and this is done between 3-5 days prior to school reopening and repeated as and when students report infestations in various rooms or dormitories. Data collected on the question “How often do you use the preferred method/approach for bed bug control?” shows firstly that, 2 respondents representing 40 % of the total respondents stated they use their preferred method/approach for 3 - 6 months. Secondly 2 respondents representing 40 % of the total respondents stated they use it for 6 months to 1 year. Lastly, 1 respondent representing 20 % opted for other, which represented no specific time frame for frequency of fumigation but it is done as at when students report infestation of bed bugs (Figure 12).

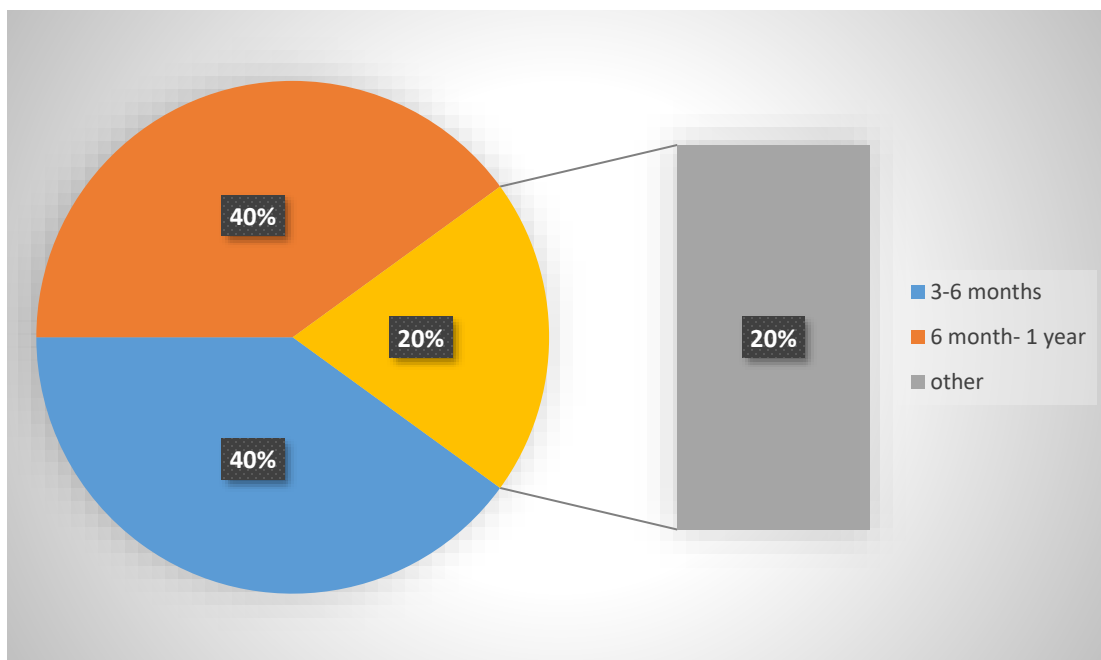


Figure 12: Frequency of fumigation

4.2.3 Products used for fumigation

From the management questionnaire on the question “If fumigation or spraying method is used, what products do you use?” Firstly, 3 respondents representing 60 % of the total management respondents stated that they use a combination of Dursban and K-Othrine super, secondly, 1 respondent representing 20 % of the total respondents stated they use Dresscare/Dress force. Lastly, 1 respondent representing 20 % stated that Pyrenix/Pyrefose was their preferred product (Figure 13).

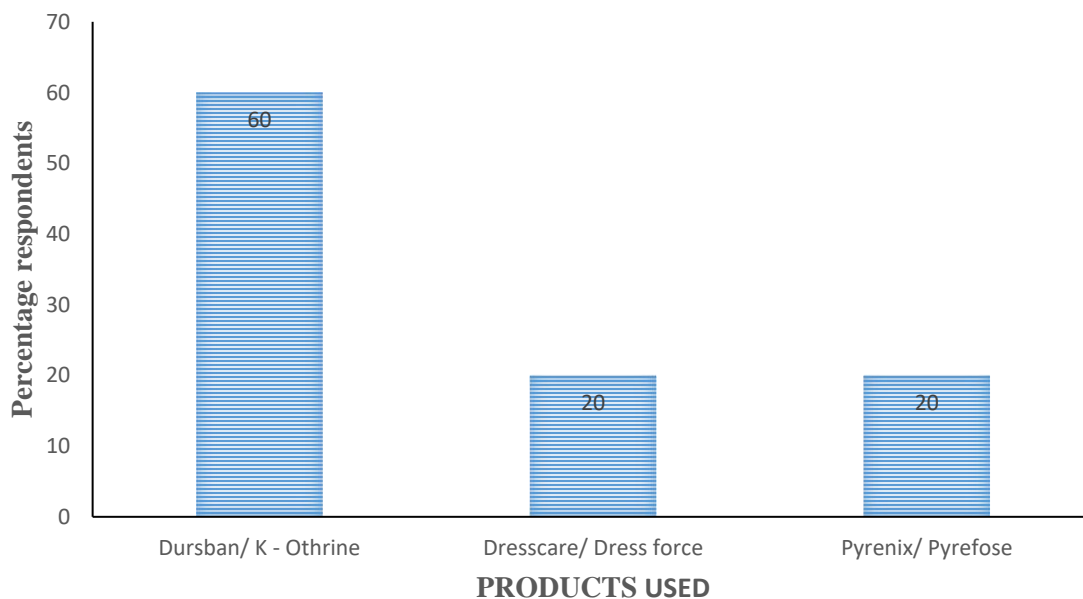


Figure 13: Products used for fumigation.

4.2.4 Duration of effectiveness of insecticide products used for fumigation against bed bugs

According to the management, the duration of effectiveness ranges from the day of application to about 4 - 6 months post-treatment of dormitories and hostels. According to the student respondents, 72 students representing 48 % stated that the treatment done by management was effective from the day of treatment to 6 months. Forty-eight students representing 32 %, stated 6 - 12 months as

the effective period, 19 representing 12.9 % stated 1 - 3 years as duration of effectiveness and 11 representing 7.1 % stated others which students further elaborated that the duration of effectiveness could be three (3) days to about two (2) weeks post-treatment.

4.2.5 Cost of controlling bed bugs in the two educational institutions

According to two (2) willing management participants on the cost of control of bed bugs, they stated that it costs sixty-one Ghana cedis (GHC 61.00) to fumigate one dormitory in one house in the Senior High School, whilst it costs fifty Ghana cedis (GHC 50.00) to fumigate each room in one hall. The cost of controlling bed bug infestation in the study sites per year is two thousand one hundred and ninety-six Ghana cedis (GHC 2,196.00) for Presec House 10 and twenty-eight thousand Ghana cedis (GHC 28,000.00) for Mensah Sarbah Hall in University of Ghana (Table 5).

Table 5: Cost of controlling bed bugs at the study sites in the educational institutions per year

<i>Parameters</i>	<i>Presec SHS (House 10)</i>	<i>Mensah Sarbah Hall, UG (Annex A, B, C and D)</i>
<i>Number of rooms</i>	18	280
<i>Size of dormitories/rooms</i>	12 m by 24 m	14 m by 14 m
<i>Number of times treated per year</i>	2	2
<i>Cost of treatment per dormitory/room using pyrethroid and organophosphate</i>	GHC 61.00	GHC 50.00
<i>Total cost per each control period</i>	GHC 1,098	GHC 14,000
<i>Total cost of treatment per year</i>	GHC 2,196	GHC 28,000

4.3 Toxicity of Insecticides to Bed bugs

A total of Two Thousand One Hundred and Forty (2,140) adult F1 generation of bed bugs were produced within a span of 3 - 5 months for used in the topical bioassay to determine the LD₅₀ and LT₅₀ of the four insecticides belonging to three classes of insecticide used in the experiment.

4.3.1 LD₅₀ of the four insecticides used in the study on *Cimex hemipterus* F1 adults

Log-dose probit mortality of bed bugs obtained by topical application of increasing doses of Chlorpyrifos, Dursban 4E, Lambda-cyhalothrin and K-Othrine Super of the two (2) populations from Presec and Mensah Sarbah Hall are shown in Table 6.

Table 6: Log-dose probit mortality data of two populations of bed bugs tested with three different classes of insecticides

Insecticide	Population	N ^a	Slope± SE	LD ₅₀ ^b µg/µl (95% CL)	χ ^{2c} (df)	P ^c
Chlorpyrifos	Presec	40	3.44±1.19	15.10 (4.75-21.03)	0.04 (1)	0.85
	M. Sarbah	40	2.17±0.39	17.48 (10.73-25.40)	1.85 (1)	0.17
Dursban 4E	Presec	40	1.88±0.62	6.00 (1.09-10.52)	0.78 (1)	0.38
	M. Sarbah	40	0.83±0.33	13.61 (0.54-40.11)	0.93 (1)	0.34
K-Othrine Super	Presec	40	1.05±0.49	4.35 (0.018-9.84)	2.51 (2)	0.29
	M. Sarbah	40	0.86±0.31	4.34 (0.881-10.55)	2.38 (2)	0.31
Lambda-cyhalothrin	Presec	40	1.34±0.34	23.26 (7.77-41.64)	0.91 (1)	0.34
	M. Sarbah	40	1.13±0.34	20.52 (2.98-42.26)	0.35 (1)	0.56

^a Overall number of insects used.

^b LD₅₀ in µg/µL per insect 24 h post-treatment.

^c Larger values of χ² for goodness-of-fit and P value < 0.05 indicate a poorer fit on the probit regression line.

N = 40

In this study, four (4) insecticides belonging to three (3) classes of insecticide were assessed for their effective lethal dose for 50 % exposed population (LD_{50}) and their median lethal time for 50 % exposed population (LT_{50}).

The concentrations 1 $\mu\text{g}/\mu\text{l}$, 5 $\mu\text{g}/\mu\text{l}$, 25 $\mu\text{g}/\mu\text{l}$ and 125 $\mu\text{g}/\mu\text{l}$ were used for topical applications but 100 % and 95 % mortality rates were achieved at all doses for K-Othrine Super and Dursban 4E and hence further dilutions to attain different concentrations were prepared for K-Othrine Super and Dursban 4E (Appendices 5 and 6).

All the insecticides demonstrated a dose-dependent insecticidal activity with Chlorpyrifos (125 $\mu\text{g}/\mu\text{l}$) recording the highest percentage mortality of 100 % and 95 % (Appendix 7) for bed bugs sampled from Presec and Mensah Sarbah Hall at the University of Ghana 24 hours post-treatment respectively. However, K-Othrine Super exhibited a significantly stronger insecticidal activity with LD_{50} values of 4.35 $\mu\text{g}/\mu\text{l}$ and 4.34 $\mu\text{g}/\mu\text{l}$ against bed bugs collected from Presec and Mensah Sarbah Hall respectively (Table 6). Dursban 4E insecticide followed next with LD_{50} values of 6.0 $\mu\text{g}/\mu\text{l}$ and 13.61 $\mu\text{g}/\mu\text{l}$ bed bugs sampled from Presec and Mensah Sarbah Hall respectively (Table 6). The Lambda-cyhalothrin insecticide showed the least potent insecticidal activity with LD_{50} values of 23.26 $\mu\text{g}/\mu\text{l}$ and 20.52 $\mu\text{g}/\mu\text{l}$ against bed bugs collected from Presec and Mensah Sarbah Hall respectively (Table 6). Population of bed bugs from the two study sites collected for testing showed different susceptibility levels to the insecticides (Table 6). The test for lack-of-fit of the probit model was not significant in all cases (Pearson chi-square test; $P > 0.05$).

Results of one-way ANOVA and Tukey post-hoc test show that there is no statistically significant difference between the insecticides and also the bed bug population areas ($P > 0.05$). A table with the summary is given in (Appendix 12).

Comparison of confidence limits indicated that the LD₅₀ values for the Presec and Mensah Sarbah Hall populations of bed bugs were significantly lower for K-Othrine Super insecticide than to any other insecticide. Significant difference did not arise between the LD₅₀s of Presec population for Dursban 4E and that of Mensah Sarbah Hall/Presec for K-Othrine Super, while both were significantly lower than population of Mensah Sarbah Hall to Chlorpyrifos.

4.3.2 LT₅₀ of the insecticides used in the study on *Cimex hemipterus* F1 adults

K-Othrine Super, a dual active-ingredient insecticide was the only insecticide that was uniformly both fast-acting and ultimately effective. Chlorpyrifos rate of effectiveness alternated from slow-acting to fast-acting but eventually effective with variation between populations in shape of the mortality curves (Appendix 10 and 11). Dursban 4E was the only insecticide that was uniformly ineffective in relation to the lethal time to kill 50 % of each population (Table 7). Lambda-cyhalothrin for Mensah Sarbah Hall was fast-acting when exposure resulted in any mortality.

LT₅₀s could not be calculated for some of the populations due to rate of efficacy being either faster than 24 hours or slower than 72 hours (Table 7). Dursban 4E has the same active ingredient as the standard used Chlorpyrifos; however Chlorpyrifos was more effective than Dursban 4E (Table 7). An LT₅₀ could not be calculated for any of the populations for Dursban 4E due to insufficient mortality after 72 hours of exposure (Table 7). K-Othrine Super recorded LT₅₀ for Mensah Sarbah Hall <24 hours (Table 7) due to extremely high initial mortality making it fast-acting and ultimately effective.

Table 7: Lethal time to kill 50 % of each population of bed bugs for insecticides used in the study

Insecticide	population	N ^a	Slope± SE	LT ₅₀ ^b HRS (95% CL)	χ^2 ^c (df)	P ^c
Chlorpyrifos 20 µg/µl	Presec	40	0.05±0.03	64.76	0.81 (9)	1.00
	M. Sarbah	40	0.01±0.02	30.55	1.21 (9)	0.99
Dursban 4E 5 µg/µl	Presec	40	0.04±0.05	> 72	2.53 (9)	0.98
	M. Sarbah	40	0.03±0.03	> 72	1.16 (9)	0.99
K-Othrine Super 5 µg/µl	Presec	40	0.02±0.05	70.83	1.15 (9)	0.99
	M. Sarbah	40	0.11±0.47	< 24	11.3 (9)	0.26
Lambda-cyhalothrin 20 µg/µl	Presec	40	0.02±0.05	> 72	2.02 (9)	0.99
	M. Sarbah	40	0.02±0.03	30.68	1.29 (9)	0.99

^a Total number of insects used.

^b LT₅₀ values were calculated using SPSS 16.0 and could not be calculated for populations with mortality greater than 90 % after 24 h (denoted as < 24) or less than 10% mortality after 72 h (denoted by > 72).

^c Larger values of χ^2 for goodness-of-fit and P value < 0.05 indicate a poorer fit on the probit regression line.

N = 40

CHAPTER FIVE

5.0 DISCUSSION

Resistance has been defined as the development of a strain capable of surviving a dose lethal to a bulk of individuals in a typical population (Brausch & Smith, 2009). Insecticide resistance is a universal phenomenon and has been recorded for most, if not all, classes of insecticides in hundreds of insect species (Karunker *et al.*, 2009). Monitoring insecticide resistance could allow one to choose the most effective product that continues to control the insect population while relieving selection pressure for resistance (Tabashnik *et al.*, 2009). Insecticide resistance is one factor that has been implicated in the recent global resurgence in populations of bed bugs, *Cimex lectularius* (Reinhardt *et al.*, 2009). In the past, populations of bed bugs were effectively and efficiently controlled with DDT and other broad-spectrum, residual insecticides (Potter, 2011). However, resistance to pyrethroid insecticides has created challenges for pest management professionals (Moore & Miller, 2009).

The purpose of this study was to document the most commonly use approaches/methods to control bed bugs in selected educational institutions, products use, frequency of use, their effectiveness, and cost involve in treatment of infestation and to document the lethal doses and lethal time of the most frequently used insecticides on the 2 populations of bed bugs used in the experiments. Dual action combination products that contain both a pyrethroid and neonicotinoid are currently some of the most effective and widely used insecticides by Pest Management Professionals (PMPs) due to their long residual activity (Potter, 2011). These commercial products stack two different modes of action with the goal of better controlling populations than either class of insecticides could alone; however, given that resistance to one of these classes (pyrethroids) has already been

extensively documented (K Reinhardt *et al.*, 2009) evolution of resistance to the combination products is of great concern.

The student survey using questionnaires asked students who lived in rooms with confirmed bed bug infestations what symptoms they associated with the bite of the bugs and their responses included itchiness, insomnia, anaemia and social stigma associated with the bite. The observation from this study relates with quite a number of studies. For example, (Potter, 2011) discovered that one survey asked individuals with confirmed bed bug infestations what symptoms they attributed to these populations other than allergic responses to the bites, and the respondents cited insomnia and various other stresses. Additionally, the emotional implications can be more severe and include delusional parasitosis and thoughts of suicide (Goddard & Deshazo, 2009).

The first issue investigated in this study was to know the control approaches/methods used in the study areas, the products used, how effective they are, duration of effectiveness and the cost of the method used. Investigation of control methods uncovered fumigation as the predominant approach/control method employed by the five (5) willing participant members of the management of the educational institutions to treat bed bug infestation in the Questioner-Interviewer Response process. The major product used and stated by the management in the interview guide questionnaire included K-Othrine Super a dual combination of pyrethroid and neonicotinoid and Dursban 4E which is an organophosphate. Currently, numerous classes of insecticides are registered for bed bug control globally (Gordon *et al.*, 2014) but insecticidal formulations containing a pyrethroid are the most commonly used products against this pest (Gordon *et al.*, 2014). Other classes including the neonicotinoids, pyrroles, desiccant dusts, insect growth

regulators, organophosphates, and carbamates are also used worldwide (Gordon *et al.*, 2014). Combination products using both of these active ingredients (pyrethroid and neonicotinoid) are ideal in the field (Potter, 2011), but the evolution of insecticide resistance in the field is a concern (Gordon *et al.*, 2014).

The dimension of dormitories at Presec (House 10) was 12 m by 24 m and that of rooms at Mensah Sarbah Hall (Annexes A, B, C and D) was 14 m by 14 m. The cost of fumigation of one dormitory in one house in the Senior High School (Presec) was recorded as sixty-one Ghana cedis (GHC 61.00) while it cost fifty Ghana cedis (GHC 50.00) to fumigate a room in one hall/hostel in the tertiary institution (Mensah Sarbah Hall; University of Ghana). Heat or gas treatment cost more than fumigation and hence adoption of the latter method. Likewise, study undertaken by Potter *et al.*, (2010) revealed that the average cost to treat a bed bug infestation in a single-family residence is \geq \$500 and to heat treat an apartment costs between \$800 – 1200 (Stedfast & Miller, 2014). Conclusively, millions of dollars are spent per year trying to control this pest in private homes (Moore & Miller, 2009). Equally, the hospitality industries spend millions of dollars on their control due to the financial impact this pest has on the destruction to brand names and products (Doggett *et al.*, 2012).

The second part of the research investigated the lethal dose of the products against two populations of bed bugs. The insecticidal activity is a measure of the killing potential of the insecticide. The probit analysis produced a confidence interval for each LD₅₀ which enabled comparison of the Confidence Intervals population overlaps.

The LD₅₀ values for some populations were significantly different and were determined by the failure of the confidence intervals to overlap (Wheeler *et al.*, 2007). Results from this study confirmed that populations collected from the field were more resistant to Chlorpyrifos and Lambda-cyhalothrin than K-Othrine Super and Dursban 4E. However, K-Othrine Super was effective at controlling vast number of the population used in the study given that it recorded the lowest lethal dose to kill 50 % of the population. This result is promising, because only K-Othrine Super contains a dual action combination of acetamiprid (neonicotinoid) and Lambda-cyhalothrin (Pyrethroid). Lambda-cyhalothrin recorded the highest lethal dose that killed 50 % (LD₅₀) of the two populations and this confirms resistance relating to work done by (Myamba *et al.*, 2002), where *Cimex hemipterus* was found to have resistance to the pyrethroids used in their study in Tanzania and also that of (Steelman *et al.*, 2008) where bed bugs *C. lectularius* used in their study were all susceptible to the pyrethroids with LC₅₀ values ranging from 0.7 to 37.1 ppm for Bifenthrin, Lambda-cyhalothrin, and Permethrin. Converting the LD₅₀ of my study to parts per million 23,260 ppm and 2,050 ppm will be recorded for Presec and Mensah Sarbah Hall respectively which is higher therefore the population could be termed as resistance. This resistance may be attributed to the use of *Cimex hemipterus* species in the present study.

In addition, pyrethroid resistant populations of bed bugs have been well documented in other studies (Yoon *et al.*, 2008), also resistance to the dual action pyrethroid/neonicotinoid products has been established in the laboratory (Gordon *et al.*, 2014). Furthermore, the underlying causes of the observed decrease in susceptibility to K-Othrine Super which is a combination product could be attributed to increase resistance to the pyrethroid constituent, but not the neonicotinoid (Gordon *et al.*, 2014). Intense levels of bed bug resistance to acetamiprid could be correlated with the

existence of various resistance mechanisms and/or severe enzyme-mediated acetamiprid metabolism (Romero & Anderson, 2016). Neonicotinoids are metabolized in several species of insects by detoxifying enzymes (Simon-Delso *et al.*, 2015). A faster enzymatic biotransformation of acetamiprid, disclosed through the manufacturing of at least five metabolites with decreased affinity to nAChRs (Simon-Delso *et al.*, 2015), may explain the elevated rates of resistance to this neonicotinoid identified in their research in bed bugs.

In this study, adult bed bugs showed high tolerance to lower concentrations of the tested insecticides to the extent that some were capable of producing eggs 48 - 72 hours post-treatment. The percentage mortality of bed bugs 24 hour post-treatment also showed that bed bugs were susceptible to higher concentrations of insecticides such as 125 $\mu\text{g}/\mu\text{l}$ for Chlorpyrifos and Lambda-cyhalothrin and 10 $\mu\text{g}/\mu\text{l}$ and 25 $\mu\text{g}/\mu\text{l}$ for K-Othrine Super and Dursban 4E respectively. Insecticidal products can be divided somewhat subjectively into two groups based on speed of action: fast-acting or slow-acting. Likewise, the relative effectiveness at killing entire populations can be divided into three categories: ultimately effective, moderately effective or ineffective (Gordon *et al.*, 2014).

Susceptibility of population at Mensah Sarbah Hall to K-Othrine Super was unique compared to all other products investigated. The LT_{50} value of population for Mensah Sarbah Hall fell below 24 hour post-treatment whilst that of Presec (House 10) population was greater than 48 hours post-treatment for the same product evaluated. Results from this study confirm that a single population of bed bugs can react differently to commercial insecticidal products comprising similar active ingredients. Some of the observed changes in susceptibility could be credited to type of

formulation. Each commercial product will contain a unique combination of inert ingredients, synergists and active ingredients that may result in diverse bioavailability to the bed bug. According to (Gordon, 2014) recently, one study found that even though Transport Mikron and Transport GHP insecticide contain the same ratio and concentration of active ingredients, the difference in formulation may affect the efficiency. In addition to formulation, the physiology of the bed bug (i.e., up-regulation of enzymes, altered target sites and changes in the cuticle) may alter the effectiveness of an insecticide (Zhu *et al.*, 2010).

Historically, populations of bed bugs of both institutions have been exposed to the products countless of time during their fumigation periods which is a minimum of 3 years and this could have contributed to the resistance level of the bed bugs to the products. Previous studies have documented resistance of adult bed bugs to combination products (Acetamiprid and Lambda-cyhalothrin) used in this study. It has also been reported that insecticide resistance is extremely variable between distinct bed bug populations. Susceptibility varied in the products used in this study on the bed bug populations. The bed bug population tested in this study were collected from different environmental sites within the Greater Accra Region and therefore, differences between resistances of bed bug to the four insecticides used could be a result of earlier insecticide exposure and selection pressure.

Behavioural resistance can be divided into two broad categories: stimulus-dependent behaviours' (e.g. irritability and repellence) and stimulus-independent behaviours (e.g. exophilic, external rest).

In the presence of insecticides, certain behavioural observations were reported in bed bugs. Higher doses of the insecticides caused increased movement and immediate paralysis, whilst some bugs did not show any sign of reaction upon contact to minimal sub-lethal doses. K-Othrine Super caused immediate paralyse of a higher number of bed bugs and rendered them unable to right themselves upon contact with sub-lethal doses of 10 $\mu\text{g}/\mu\text{l}$ and 5 $\mu\text{g}/\mu\text{l}$. For concentrations 5 $\mu\text{g}/\mu\text{l}$ for Chlorpyrifos and Lambda-cyhalothrin, 1.5 $\mu\text{g}/\mu\text{l}$ for Dursban 4E and 0.5 $\mu\text{g}/\mu\text{l}$ for K-Othrine Super bed bugs movement increased for a few seconds upon contact and right themselves from overturned position back in a minute or two. Bed bugs did not show any avoidance behaviour when exposed to lower doses of all the insecticides.

These observations conform to results of the study undertaken by (Dang *et al.*, 2017). From their results, it has been disclosed that insecticide-susceptible and insecticide-resistant *Cimex lectularius* can either prevent resting on filter paper treated with deltamethrin or increase their motion after direct contact with deltamethrin sub-lethal doses. This may have suggested excito-repellence, a form of resistance to behaviour. However, in another research, Moore & Miller discovered that *Cimex lectularius* insecticide-susceptible and insecticide-resistant strains did not avoid Lambda-cyhalothrin-treated surfaces. When subjected to chlorfenapyr, *Cimex lectularius* also showed no avoidance behaviour. Bed bugs are very cryptic and often hide in dark regions, cracks and crevices. This outstanding behavioural feature can safeguard them from contacting or finding insecticide on treated surfaces during the inspection phase.

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS AND LIMITATIONS OF THE STUDY

6.1 Conclusion

Findings of this study revealed that fumigation with insecticides is the most common approach/method used to control bed bugs. The products used mostly constitute active ingredients such as pyrethroid, neonicotinoid and organophosphate. It was also revealed that the average cost of using this approach/method in bed bug infestation treatment and control is high.

It may be concluded that the efficacy of dual action mode insecticide was higher than the single action mode insecticides for the adult stages of the bed bugs used in this study. The different bed bug populations from the two study sites showed different tolerance levels towards the insecticides and this could be a result of earlier insecticide exposure over the years of fumigation. In terms of doses, both populations were susceptible to higher doses and also insects showed higher percentage of mortality to the higher doses. As shown in this research by the relatively small LD₅₀ of bed bugs to the dual combination product, it would be indiscreet to suggest that pyrethroids and neonicotinoids are ineffective in field application to prevent bed bugs from being controlled. Relatively speaking, each individual control condition will probably dictate the choice of insecticide, how it is applied and the amount of control provided by various embedded strategies employed by a professional pest management. Survival of bed bugs after insecticide application showed that the pest may not be readily eradicated owing to modified mechanisms or resistance development. Therefore, to explore the true reasons for their resilience, more research is needed.

6.2 Recommendations

During the course of this study, some observations were made, which will require additional study to reach a suitable conclusion. Hence the following recommendations are research areas that could be exploited:

- The use of synergists, products containing novel modes of action or multiple active ingredients stacked in combination with synergists may be necessary to help control populations of bed bugs.
- Studies on bed bugs be carried out with different species and wider sample collection sites (example: habitations such as hotels, human residence etc.) across the country to further investigate their resistance level to wider range of common insecticides used by management teams.
- Molecular studies should be carried out in bed bug populations to further establish reasons for their resistance to majority of classes of insecticides.

6.3 Limitations encountered during the study

- It was difficult to get the number of bed bugs needed to be used for all four replications of all the insecticide bioassays simultaneously. Bioassays therefore had to be performed in several batches depending on the accessibility of bed bugs.
- Establishing a colony was also difficult, as acclimatizing to the conditions in the insectary was a challenging task for the field bed bugs.

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APPENDICES

Appendix 1

Management Interview guide

As part of my MPHIL research thesis at the University of Ghana, I am conducting a study that investigates the insecticide resistance status of bedbugs to target products on the Ghana market. “I will appreciate if you could complete the following questions. Any information obtained in connection with this study will be kept anonymous.

1. Is there bed bug infestation in the school/hostel/hall? YES/NO

2. How long have you known about the infestation?

0 – 6 months 6 – 12 months 1 – 3 years Others

If Others then state:

3. What control methods/approaches do you use?

4. How often do you use the methods/approaches mentioned above?

5. Has there been any positive outcome of using the method/approach? YES/NO

6. If fumigation or spraying method is used, what products do you use?

7. How do you get the products for fumigation/spraying?

8. Is/Are the product(s) effective at reducing the bed bug numbers? YES/NO

9. What is the duration of effectiveness of the product(s)?

0 – 6 months 6 – 12 months 1 – 3 years Others

If Others then state:

10. Has any side effect of product(s) been reported? YES/NO

If 'YES' please name some

11. What is the cost of controlling the bed bugs per each control period?

12. What are the challenges in controlling the bed bugs?

13. Any recommendations to improve control of the bed bugs?

Appendix 2

Student Questionnaire

As part of my MPHIL research thesis at the University of Ghana, Legon, I am conducting a study that investigates resistance status of bed bugs to target insecticide products on the Ghana market. I will appreciate it if you could complete the following table. Any information obtained in connection with this study will be kept anonymous.

YES NO COMMENTS

Do you have any knowledge on bed bugs?

Have you been bitten by bed bugs before?

Was there an effect of the bite on you?

Which of the following effect of the bite did you experience?

Itchiness Insomnia/Sleeplessness Anaemia Social stigma associated with bite

Others

If others then state:

How long have you known about the presence of bed bugs in your room/hostel?

0 – 6 months 6 – 12 months 1 – 3 years others

If others then state:

YES NO COMMENTS

Do you have any knowledge on the control of bed bugs?

Are any of the control used in any of your room/hostel?

Are they effective?

What is the duration of effectiveness?

0 – 6 months

6 – 12 months

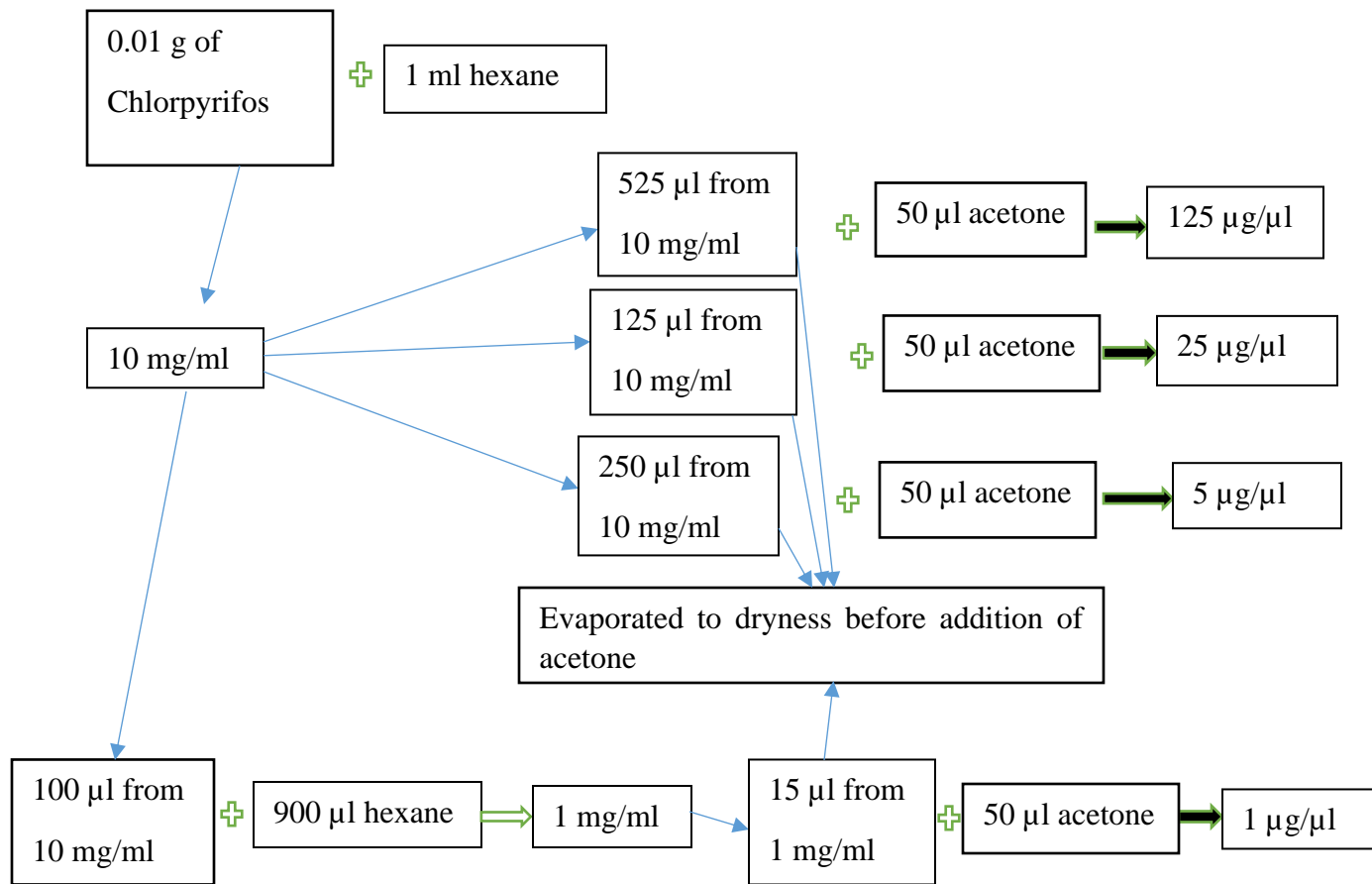
1 – 3 years

Others

Please state if others:

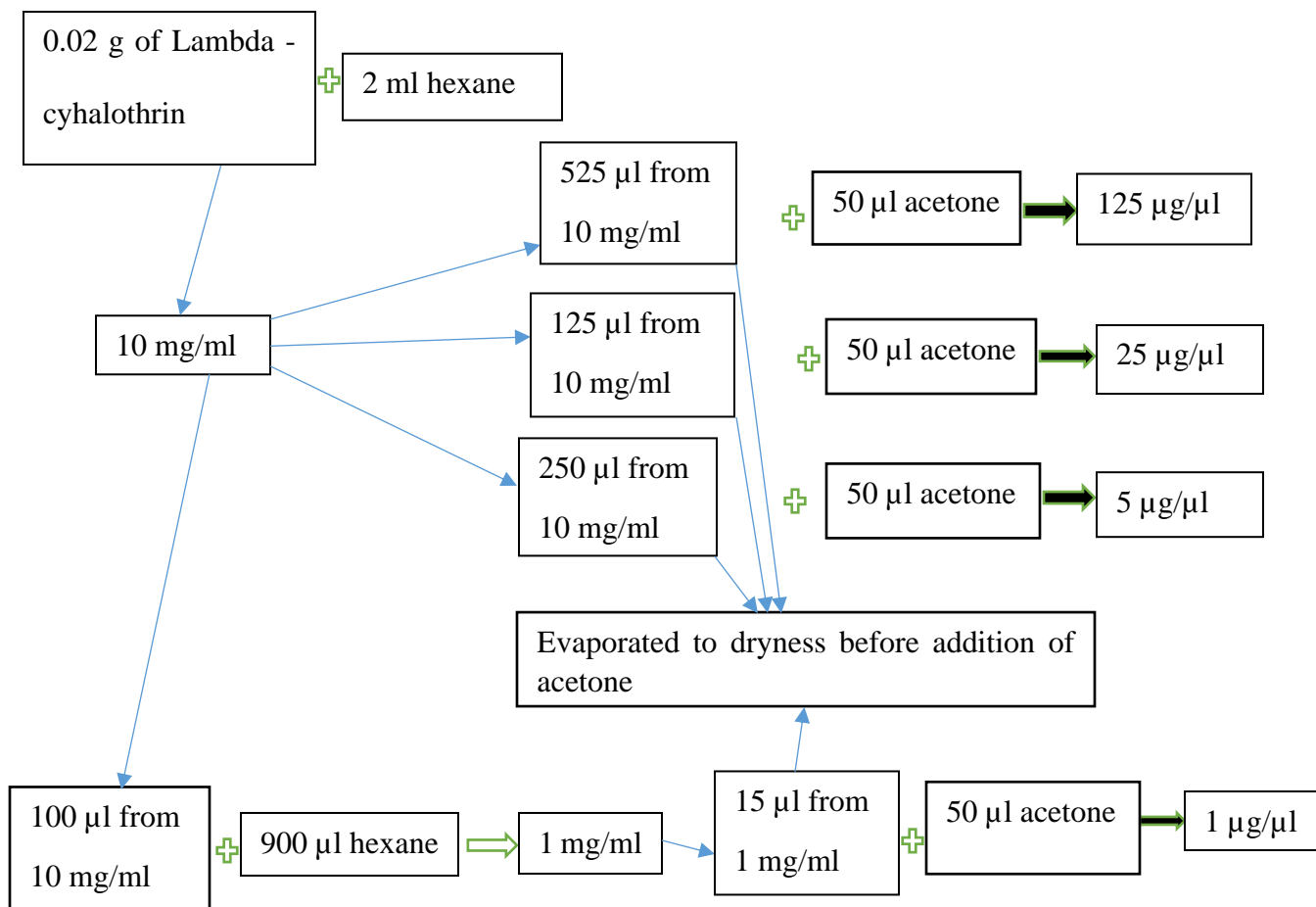
Appendix 3

A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the Chlorpyrifos insecticide in this study.



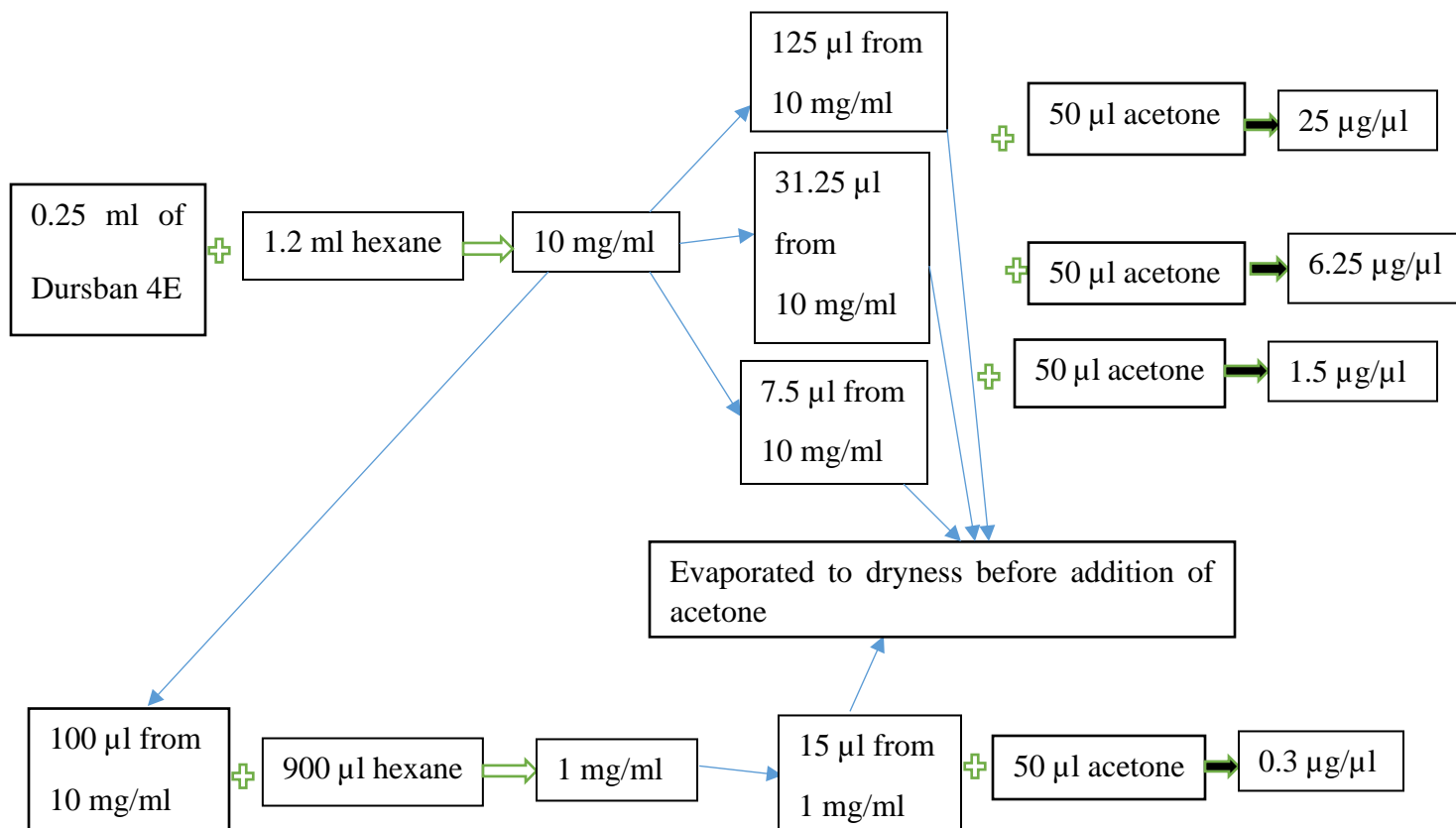
Appendix 4

A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the Lambda–cyhalothrin insecticide in this study.



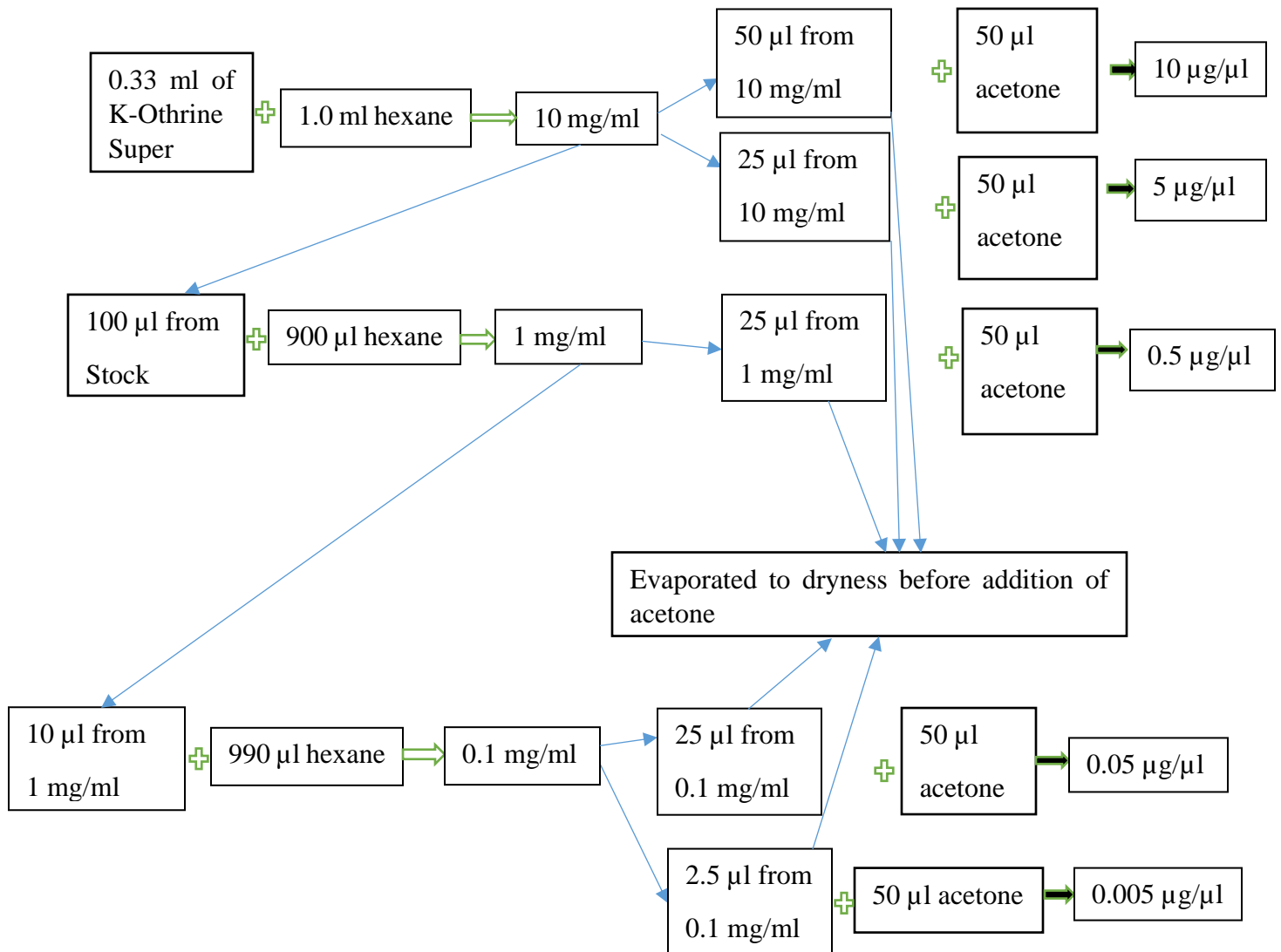
Appendix 5

A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the Dursban 4E insecticide in this study.



Appendix 6

A flow chart showing the preparation of stock solution and serial dilutions of the various concentrations used for the K-Othrine Super insecticide in this study. Sample taken from



Appendix 7

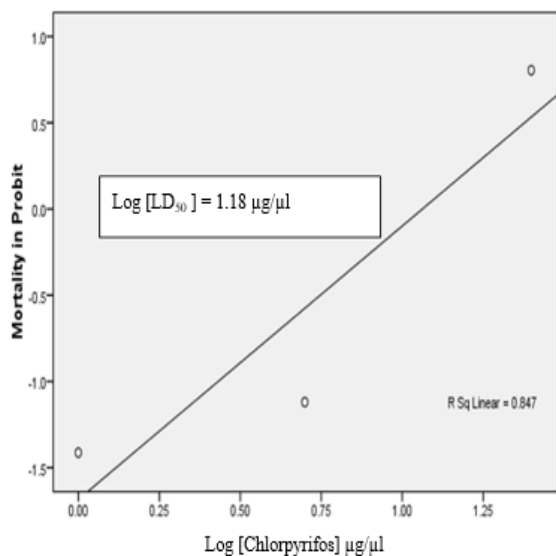
Percentage of adult bed bugs mortality for both study areas 24 hours post-treatment

Insecticides	Population	Concentration ($\mu\text{g}/\mu\text{l}$)	Percentage mortality beg bugs (%)
Chlorpyrifos	Presec	Low 1	12.5
		High 125	100
	Mensah Sarbah	Low 1	10
		High 125	95
Dursban 4E	Presec	Low 0.3	25
		High 25	92.5
	Mensah Sarbah	Low 0.3	7.5
		High 25	55
K-Othrine Super	Presec	Low 0.005	15
		High 10	77.5
	Mensah Sarbah	Low 0.005	7.5
		High 10	72.5
Lambda-cyhalothrin	Presec	Low 1	10
		High 125	82.5
	Mensah Sarbah	Low 1	7.5
		High 125	80

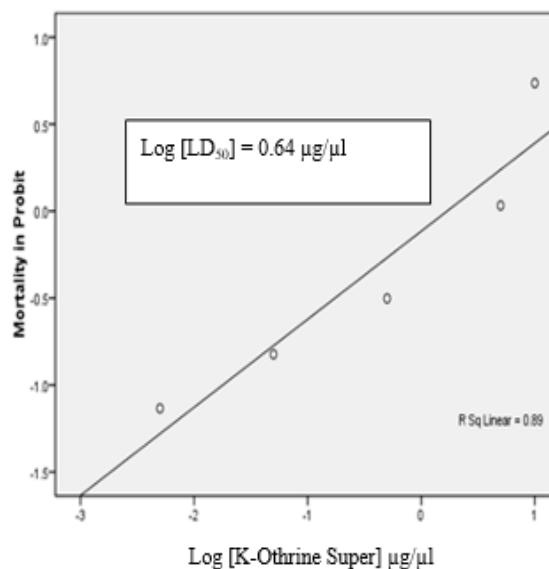
Appendix 8

Insecticidal activity of (A) Chlorpyrifos, (B) K-Othrine Super, (C) Lambda-cyhalothrin and (D) Dursban 4E on bed bugs collected from Presec. The insecticidal activity was determined at 125 $\mu\text{g}/\mu\text{l}$ for Chlorpyrifos, K-Othrine Super at 10 $\mu\text{g}/\mu\text{l}$, Lambda-cyhalothrin at 125 $\mu\text{g}/\mu\text{l}$ and Dursban 4E 25 $\mu\text{g}/\mu\text{l}$.

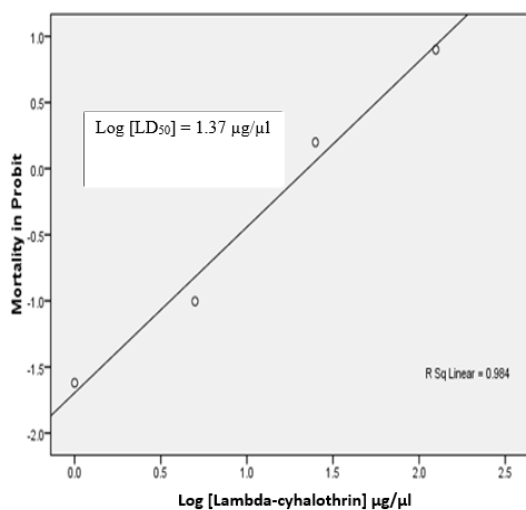
A



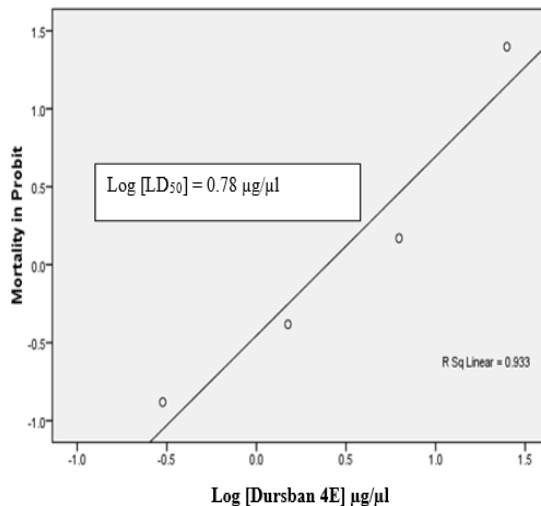
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C



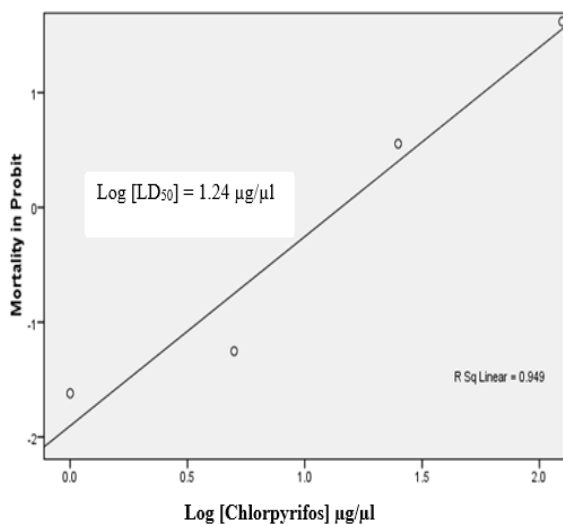
D



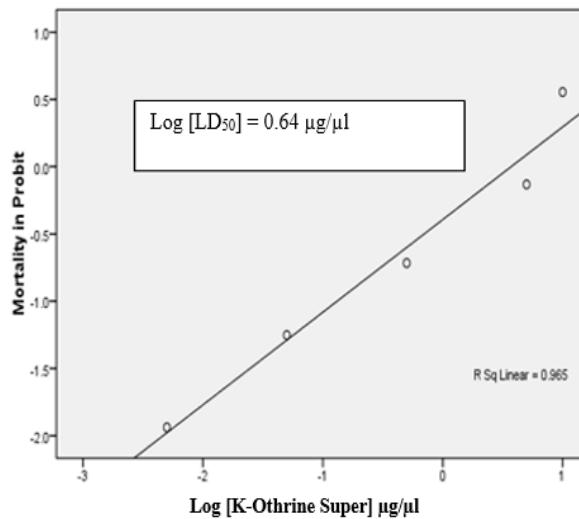
Appendix 9

Insecticidal activity of (E) Chlorpyrifos, (F) K-OthrineSuper, (G) Lambda-cyhalothrin and (H) Dursban on bed bugs collected from Mensah Sarbah Hall. The insecticidal activity was determined at 125 $\mu\text{g}/\mu\text{l}$ for Chlorpyrifos, K–Othrine Super at 10 $\mu\text{g}/\mu\text{l}$, Lambda-cyhalothrin at 125 $\mu\text{g}/\mu\text{l}$ and Dursban 4E 25 $\mu\text{g}/\mu\text{l}$.

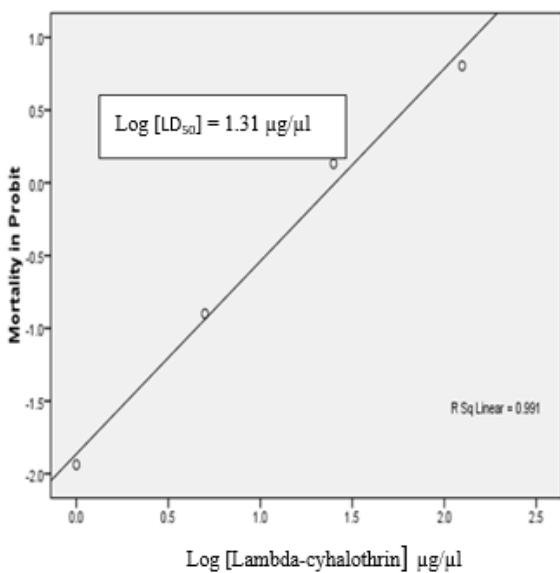
E



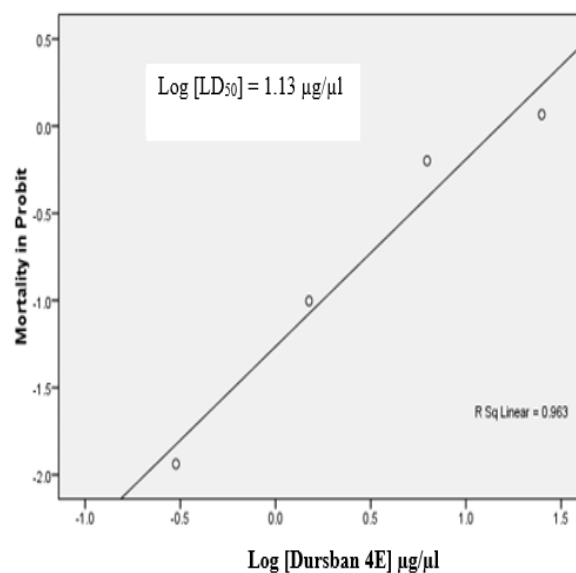
F



G

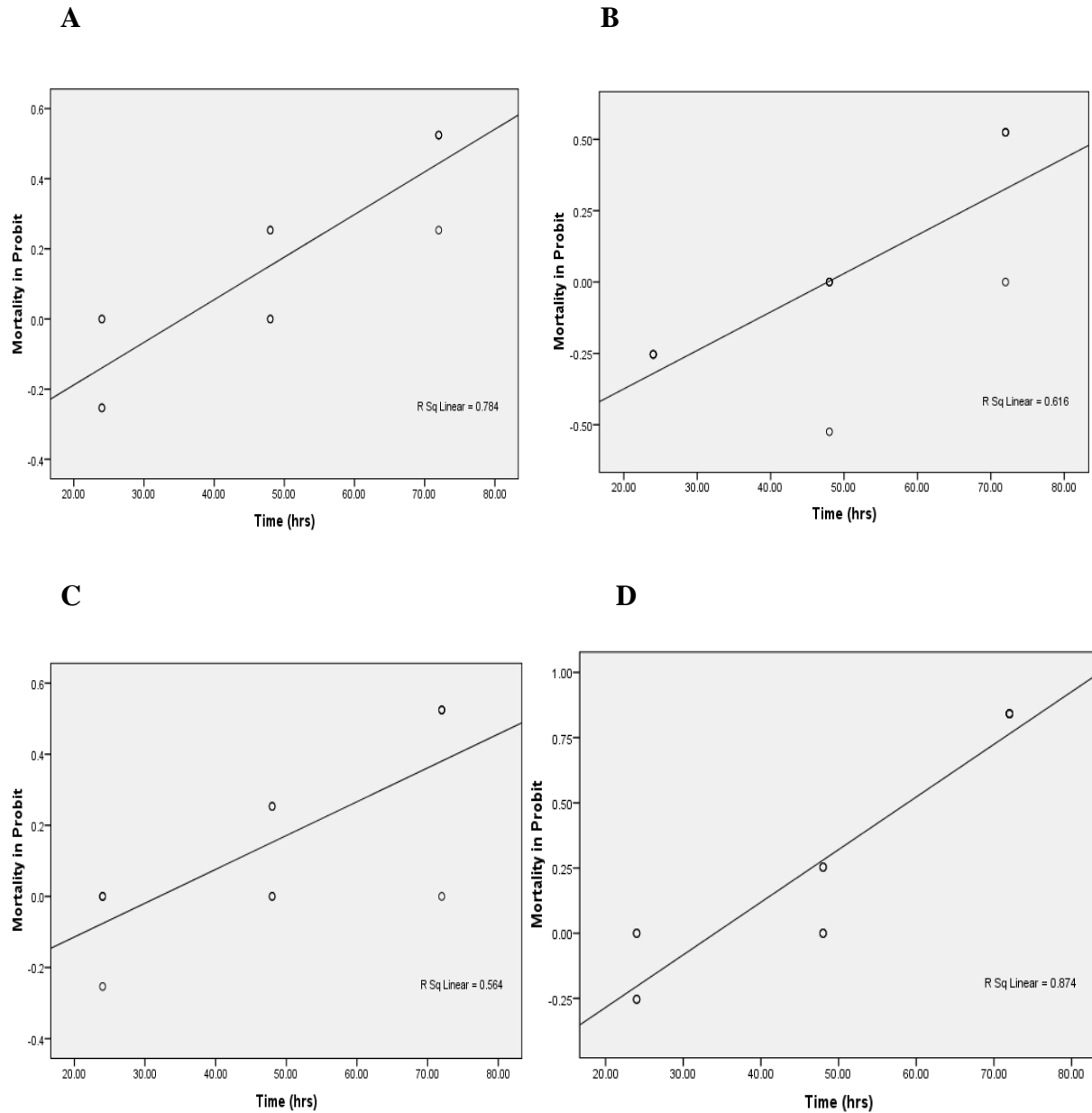


H



Appendix 10

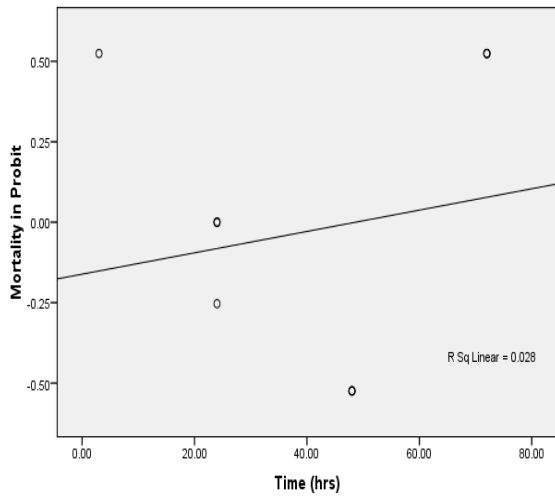
Mortality over time of one population of bed bugs collected from Presec to 4 commercial products approved for bed bug control: graph A represents K-Othrine Super, graph B represents Dursban 4E, graph C represents Lambda-cyhalothrin and graph D represents Chlorpyrifos.



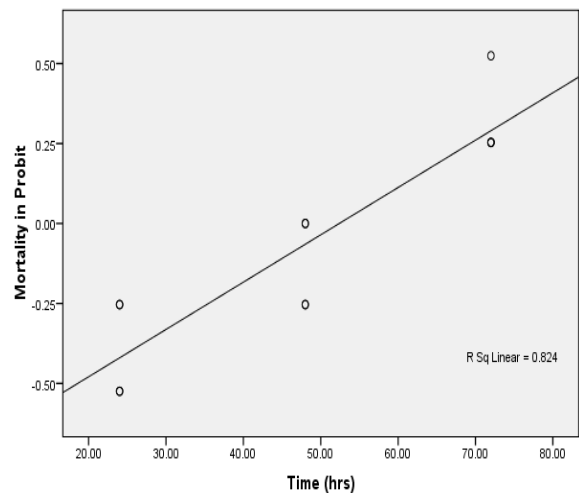
Appendix 11

Mortality over time of one population of bed bugs collected from Mensah Sarbah hall to 4 commercial products approved for bed bug control: graph E represents K-Othrine Super, graph F represents Dursban 4E, graph G represents Chlorpyrifos and graph H represents Lambda-cyhalothrin.

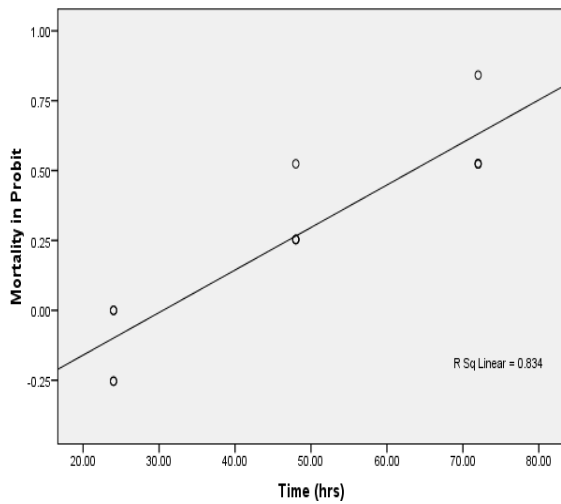
E



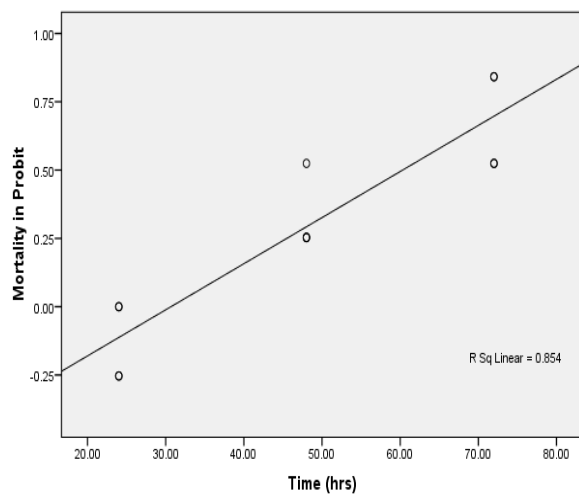
F



G



H



Appendix 12

T-Test

[DataSet1] C:\Users\Abankwa\Desktop\MADAM EVA\LT SHORTCUT FOR ALL.sav

Group Statistics

Area	N	Mean	Std. Deviation	Std. Error Mean
Concentration Presec	4	12.1875	8.77552	4.38776
M. Garbah	4	13.9875	7.02585	3.51293

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Concentration	Equal variances assumed	.586	.473	-.320	6	.760	-1.80000	5.62077	-15.55353	11.95353
	Equal variances not assumed			-.320	5.726	.760	-1.80000	5.62077	-15.71469	12.11469

Multiple comparison for insecticidal activities on bed bugs collected at Presec

Multiple Comparisons

Dependent Variable: Concentration

	(I) Insecticide	(J) Insecticide	Mean Difference (I- J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Dursban	K- Orthrine	1.13400	8.18098	.999	-25.0644	27.3324
		Lambda	-18.35333	8.18098	.191	-44.5517	7.8450
		Chlorpyrifos	-7.77667	8.18098	.780	-33.9750	18.4217
	K- Orthrine	Dursban	-1.13400	8.18098	.999	-27.3324	25.0644
		Lambda	-19.48733	8.18098	.158	-45.6857	6.7110
		Chlorpyrifos	-8.91067	8.18098	.705	-35.1090	17.2877
	Lambda	Dursban	18.35333	8.18098	.191	-7.8450	44.5517
		K- Orthrine	19.48733	8.18098	.158	-6.7110	45.6857
		Chlorpyrifos	10.57667	8.18098	.592	-15.6217	36.7750
	Chlorpyrifos	Dursban	7.77667	8.18098	.780	-18.4217	33.9750
		K- Orthrine	8.91067	8.18098	.705	-17.2877	35.1090
		Lambda	-10.57667	8.18098	.592	-36.7750	15.6217

Multiple comparison for insecticidal activities on bed bugs collected at Mensah Sarbah Hall

Multiple Comparisons

Dependent Variable: Concentration

	(I) Insecticide	(J) Insecticide	Mean Difference (I- J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Dursban	K- Orthrine	12.82967	12.05263	.719	-25.7671	51.4264
		Lambda	-3.83333	12.05263	.988	-42.4301	34.7634
		Chlorpyrifos	.21667	12.05263	1.000	-38.3801	38.8134
	K- Orthrine	Dursban	-12.82967	12.05263	.719	-51.4264	25.7671
		Lambda	-16.66300	12.05263	.542	-55.2598	21.9338
		Chlorpyrifos	-12.61300	12.05263	.729	-51.2098	25.9838
	Lambda	Dursban	3.83333	12.05263	.988	-34.7634	42.4301
		K- Orthrine	16.66300	12.05263	.542	-21.9338	55.2598
		Chlorpyrifos	4.05000	12.05263	.986	-34.5468	42.6468
	Chlorpyrifos	Dursban	-.21667	12.05263	1.000	-38.8134	38.3801
		K- Orthrine	12.61300	12.05263	.729	-25.9838	51.2098
		Lambda	-4.05000	12.05263	.986	-42.6468	34.5468