MOLECULAR CHARACTERIZATION AND PATHOGEN LOADS ASSESSMENT OF *Cimex spp.* (BEDBUGS) IN SELECTED COUNTIES OF KENYA

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Molecular Characterization and Pathogen Loads Assessment of *Cimex Spp.* (Bedbugs) in Selected Counties of Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my mom, sister and brothers

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LIST OF ABBREVIATIONS AND ACRONYMS

ASCII	American Standard Code for Information Interchange
AUC	Area Under Curve
BLAST	Basic Local Alignment Search Tool
DNA	Deoxyribonucleotide Acid
Kdr	Knockdown Resistance
MgCL	Magnesium Chloride
mtDNA	Mitochondria Deoxyribonucleotide Acid
MUSCLE	Multiple Sequence Comparison by Log-Expectation
NaoCL	Sodium Hypochlorite
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographical Information System
ROC	Receiver Operating Characteristic Curve
WHO	World Health Organization

ABSTRACT

Bedbugs, voracious blood-feeding ectoparasites, are indeed a potentially significant health problem within resident communities in Kenya. However, there is paucity of available studies on epidemiological or clinical data about the causal relationship between human beings and bed bugs. This study was carried out across selected infested counties in Kenya. The aim of this study was to identify, characterize, and assess pathogen loads for sustainable management of bedbugs in Kenya. A field survey was conducted in nine counties of Kenya namely, Nairobi, Mombasa, Kisumu, Bomet, Baringo, Narok, Machakos, Makueni and Kiambu using a semi-structured questionnaire. Nine hundred respondents (100 per county) were interviewed while five individual bedbug samples were used for genomic DNA extraction. Maximum entropy distribution modeling (MaxEnt) was used to map and predict the potentially suitable habitat, while Vensim PLE 8.0.9 software was used to implement Cimex spp. system dynamics models and carry out simulations. Field survey results revealed the socio-demographic profile of the respondents (females = 49.8%; males = 50.2%; minimum age = 18 years; maximum age = 96; mean age = 38.15 year), perceptions and incidence in the communities (weird occurrence = 80%; most active at night = 97%; mild bites = 68.9%; severe bites = 28.8%; disinfected more than once = 89.8%; present in bedroom/mattresses = 34%; furniture = 28%; cracks/crevices =24%; clothes =14%) and bedbug management practices (use of both chemical and cultural practices = 79.3%; cultural only = 10.3%; chemical only = 9.2%; botanicals = 1.1%). Morphological examination, confirmed through MtCOI gene sequencing, revealed that the collected bedbugs belonged to the Cimex genus. Further, molecular identification revealed that the examined samples belonged to two different species: *Cimex hemipterus* (n = 24; 67.7%) and *Cimex lectularius* (n = 12; 33.3%). The jack-knife test demonstrated that land cover and Temperature Seasonality (bio_4) are the most important variables in determining the suitability of bedbug in Kenya while Precipitation of Driest Month (Bio_14) and Precipitation of Warmest Quarter (Bio_18) variables significantly contribute to determining its suitability in Africa. Insecticide resistance was confirmed through VGSC gene sequencing revealing 79 (28.5%) of the 277 screened samples had the resistance gene. The total gut microbiome library size was 675,130 with *Wolbachia* (68.42%), *Klebsiella* (4.90%) and *Escherichia* (3.31%) detected as the predominant microbes. *Wolbachia* was the most predominant microbe in both of the species: *Cimex hemipterus* (70.72%) and *Cimex lectularius* (62.67%). A wide range of pathogenic genera including *Escherichia* (3.31%), *Salmonella* (0.72%) and *Yersinia* (0.45%) were detected in bedbugs. The results demonstrate that the pest is a global community ordeal, therefore these key study findings form a critical basis for designing, monitoring and implementing imperative control strategies with an ultimate goal of curbing the wide spread of the pest. This study therefore recommends integrated pest management (IPM) strategy by combining different control methods should be a better intervention approach in control of bedbugs as it also limits dependence on pesticides and reduces risks for humans and the environment.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Bed bugs are small, oval, brownish insects that are strictly hematophagous (Akhoundi *et al*., 2015). Adult bed bugs are approximately 5mm in length, reddish brown, wingless, dorsoventrally flattened and resembling unfed ticks and cockroach nymphs (Goddard, 2008). They give an off-odor similar to that of rotting strawberries serving as a means of detection. Their nymphs range from 1-4mm approximately, yellowish white in color and translucent (Goddard, 2008). They don't usually fly but can move quickly over floors, walls and ceilings.

A bed bug's midgut microbiome/microbiota simply represents the collective genome of the micro-organisms residing in that particular environmental niche or the micro-organisms themselves respectively. The gut microbiota is a major factor that determines pathogen and infection transmission. On the other hand, insecticide resistance is "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (Dang *et al*., 2017a). The common bedbug has got several resistance mechanisms ranging from knockdown resistance by kdr mutations, penetration resistance by remodeling and evolving of a thicker cuticle, metabolic resistance through upregulation of detoxification enzymes (e.g., ABC transporters, esterase's, and P450s) (Dang *et al*., 2017a; Zhu *et al*., 2016).

Bed bugs feed on human blood and probably are the most common ectoparasites on people worldwide due to the spread of human activities (Reinhardt & Siva-Jothy, 2007). In addition, their infestation occurs across all socioeconomic levels and ethnic groups hence their drastic increase. They usually pierce using their rostrum, a straw like structure with a pointed end. Areas affected by their bites are normally the exposed body parts including legs, hands, arms shoulders, neck and face. The bites can be in a zigzag or in a line depending on the individual's reaction ability to the

saliva. They are primarily nocturnal, feeding mainly between midnight and 5 am by detecting the levels of carbon dioxide when asleep (Doggett *et al*., 2012b). Bed bugs are really problematic and highly cryptic with an ability to hide from contacting treated surfaces and evade inspection process by hiding in cracks and crevices (Zhu *et al*., 2013).

According to United States Environmental Protection Agency, bedbugs are reported to carry more than 40 microorganisms in their saliva, stomach, exoskeleton and feces therefore are a major global health concern (USEPA, 2015). They have been reported from 135 countries in 5 continents (Akhoundi *et al*., 2019). Among them include: Asia, North America, South America, Australia and Africa. Furthermore, some of the African countries that have reported bedbug prevalence include: Nigeria with 50% from homes and school hostels (Okwa & Omoniyi, 2016) and Sierra Leone with 98% in internally displaced camps (Gbakima *et al*., 2002). Several factors have been linked to their drastic increase among them being international travels at 1.1 billion in 2014 and an estimation of 1.8 billion by 2030 translating to high levels of detection and infestations in hotels, aircrafts, trains and boats (Delaunay & Pharm D, 2012).

Over the past decade, bed bugs have resurged in many parts of the world mostly due to increase in international travels, frequent exchange of secondary items, inadequate pest control and insecticide resistance (Dang *et al*., 2017a). Moreover, unsanitary conditions, household nature and structure of traditional houses provides an ideal environment for their survival (Myamba *et al*., 2002). Nevertheless, their resurgence has largely been associated with insecticide resistance (Romero *et al*., 2007b). Several chemical formulations among them being the use of fumigants, powder dusts and other liquids against bed bugs have been availed in the recent past (Naylor & Boase, 2010). Organochloride dichloro-diphenyl trichloroethane (DDT) was the first inexpensive modern insecticide to have been discovered for the control of insect pests among them bed bugs but is discouraged due to its deleterious environmental effects. Nonetheless, non-chemical control and management methods such as vacuuming, heat treatment and removal of the already infested items has been advocated. (Doggett *et al*., 2012a).

Additionally, bedbug resurgence has been reported around the world, that is in Australia, Canada, USA and Africa (Davies *et al*., 2012a). Kenya has not been spared based on a 2015 media report where residents of Nakuru county from about 4000 homes were found to be heavily infested by bedbugs (Samuel *et al*., 2020). Therefore, the aim of this study was to assess the current management approaches and socio-economic impacts of bedbug infestations in households, determine their genetic diversity, establish insecticide resistance through detection of kdr mutation (s) and establish pathogen loads in the gut microbiome using 16S metagenomics. The key study findings form a critical basis that can be used by the state and other relevant departments to intercede, design, monitor, implement control strategies and provide assistance in creating novel information avenues with an ultimate goal of curbing the wide spread of the parasite.

1.2 Statement of the problem

The annual increase of bed bugs globally has been estimated to be 100-500% posing a great danger (Bai *et al*., 2011). The female bedbug can lay about 200-500 eggs (Delaunay *et al*., 2011) which increases chances of spread if proper management measures are not taken. They are a socioeconomic burden by the fact that they are highly mobile. In most developing countries, the infestation is a real menace with an approximate of USD 2500 - USD 3000 per infestation spent in disinfection and replacing infested belongings in households using a standard insecticide (Davies *et al*., 2012b). It is even worse in commercial and industrial places costing more millions of dollars.. Their bites have adverse side effects ranging from mild to severe. Approximately 70% of the bites result to allergic related effects including urticaria, itchmen erythematous and papular lesions associated with itch (Rahim *et al*., 2016). Scratching of the bites can lead to secondary bacterial infections such as lymphangitis, ecthyma, impetigo and cellulitis (Koganemaru & Miller, 2013). Mental health effects such as paranoia, insomnia, emotional distress and anxiety have as well been reported by residents in the infested areas (Davies *et al*., 2012b). Bedbug infestations not only result to a bad reputation and high cost of disinfection of affected areas such as hotels but also is a great threat to human health and to local economy due to decreased levels of tourism (Cambronero *et al*., 2020). Their

infestation in poultry has also resulted into anemia which in turn leads to low egg production (Sato et al., 2019). A study by (Tawatsin *et al*., 2011a) showed that bedbugs also harbor antibiotic resistant bacteria (*Enterococcus faecium* and *Staphylococcus aureus),* a great public health concern. Their pathological effects include urinary tract infections, endocarditis, bacteremia, intra-abdominal infections (IKE, 2017) and pneumonia, osteomyelitis, cellulitis (Kobayashi *et al*., 2015) respectively. Kenya is among the countries experiencing the global resurgence of bedbugs (Samuel *et al.*, 2020).

1.3 Justification of the study

The high levels of resurgence coupled with insecticide resistance necessitate an urgent need for novel, effective and efficient strategies to manage the nuisance and curb the notorious pest. The pests have also been suggested as possible vectors of pathogens having shown the ability to transmit *Trypanosoma cruzi* (etiological agent of Chagas disease) and *Bartonella quintana* (etiological agent of trench fever) (Sentana-Lledo *et al*., 2016). However, there is limited information for their ability to transmit other pathogens to humans such as hepatitis C virus and *Penicillin chrysogenum*. On the other hand, other *Cimicidae* family members have been confirmed to be competent vectors for arboviruses in birds (wild bats) (Adelman *et al*., 2013). Insecticide resistance has been pointed out as the main contributing factor to global resurgence of the pest (Romero *et al*., 2007b). Therefore, the same reason could not be ruled out from being behind the recently reported infestations and resurgence in Kenya and Africa. However, there is paucity of available studies on epidemiological or clinical data about the causal relationship between human beings and bed bugs. The aim of this study was to bridge the gap by understanding the infestation dynamics of bedbugs in households and the related socio-economic impacts which will contribute to the realization of vision 2030.

1.4 Research hypotheses

1.4.1 Null hypothesis

i. Bedbug infestation has not led to socio-economic effects among those infested.
ii. There is no genetic diversity of bedbugs infesting Kenyan population.
iii. Bedbugs have not developed insecticide resistance.
iv. Bloodmeal would not affect microbiome composition, therefore it does not contribute in their ability to transmit pathogens.

1.5 Objectives

1.5.1 General objective

The general objective of this study was to identify, characterize and assess pathogen loads for sustainable management of bedbugs in Kenya.

1.5.2 Specific objectives

1. To assess the current management approaches and socio- economic impacts of bed bug infestations in households.
2. To identify, assess the genetic diversity, polymorphism and population structure of bedbugs in Kenya using the mtCO1 gene.
3. To establish insecticide resistance of bedbugs through detection of kdr mutation site(s) on the VGSC gene target.
4. To establish pathogenic loads in the gut microbiome of bedbugs using 16S metagenomics.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and distribution of bedbugs

Bedbug association with human beings is believed to have started when they lived in caves of Middle East and Europe during the Pleistocene, Paleolithic, and Neolithic periods (Koganemaru & Miller, 2013). The two most important species of these ectoparasites are the common (or temperate) bedbug, *Cimex lectularius* (Linnaeus 1978) and the tropical bedbug, *Cimex hemipterus* (Fabricius, 1803) which occasionally parasitize domesticated animals and birds, such as chicken and bats (Davies *et al*., 2012b). However, they mostly affect human beings (Puttgen KB., 2014). Bedbug infestations were first documented in Grece by 400 B.C, Italy in 71 A.D, China in 600 A.D, Germany in the 11th century, France in the 13th century, England by late 1500s, and North America in the 1600s (Koganemaru & Miller, 2013). Additionally, they were reported in the UK, Sweden, Norway, Denmark, Australia, Switzerland, and USA (Tawatsin *et al*., 2011b). Besides, Africa was not spared by the current wave of resurgence having been reported in countries such as Nigeria (Okwa & Omoniyi, 2016), Sierra Leone (Gbakima *et al*., 2002), Tanzania (Boase, 2001), and Kenya (Samuel *et al*., 2020). The global resurgence of bedbugs has been attributed to various factors including immigration, lack of awareness in their management, stigma related to reporting, growth of international travel, compromised pesticide efficacy, insecticide resistance, and negligence of insecticide use due to toxicity concerns (Wang & Wen, 2011). Further, inaccessibility of quality information regarding the efficacy of the control products, laxity of the regulatory authorities in ensuring the effectiveness of the marketed products against the current field strains, slow response in the development of bed bug control management standards by the pest management industry association and lack of training to the pest control technicians in eradicating insecticide resistance in bedbugs have as well been cited as contributing factors for the current resurgence (Doggett *et al*., 2011).

Bedbug infestations have been reported in the urban environment, including college dormitories, hotels, health care facilities, apartments, rooming houses, and single-

family dwellings (Hwang, Svoboda, De Jong *et al*., 2005). This has been facilitated by various pathways of dispersal that can be grouped as either passive or active (Wu *et al*., 2014b). Passive means involves human movement where the pest is carried by the host and their effects such as clothing, luggage, and furniture, while active spreading involves the pest crawling between apartment units via shared infrastructure (Wu *et al*., 2014b). Furthermore, bedbug nesting sites can be classified as permanent or temporary. Permanent nesting sites comprise premises where people have established a long-term residence such as condominiums, apartments, and homes. In contrast, temporary ones include transportation venues (taxi stands, airports, bus stations), personal effects, and luggage (Kells, 2006a). Bedbugs usually hide in harbourages such as cracks and crevices of wooded furniture, beds, wall voids, under-fitted carpets, behind pictures, skirting boards and headboards, seams on mattresses, in bed-frames, inside electrical fittings, and in curtains (Boase, 2001). However, this nuisance pest can infest almost everywhere people frequently reside. Bedbug infestations are a global community spanning ordeal, thus a pertinent issue of concern.

2.2 Economic impacts of bedbug infestations

Bedbug infestation affects all socioeconomic backgrounds, which comes along with financial distress. This has led to serious economic consequences as a result of being a socioeconomic burden in society. The disinfestation process has been pointed out as an expensive exercise since it involves constant inspection, quarantine of infested areas, treatments, disposal, and replacement of infested household items as well as other furnishings (Romero *et al*., 2007b). Past studies have reported serious economic consequences, especially in the hospitality and tourism sectors (Seidel & Reinhardt, 2013). In 2010, a survey in the United States reported that US$800 to US$1,200 were used for disinfestation for every infested area (Zhu *et al*., 2016), while in Australia, an estimate of AUS$100 million was used in 2006 (Doggett *et al*., 2011).

Furthermore, it was approximated that a cost amounting to USD 2500 – USD 3000 would be spent in disinfection and replacement of infested belongings using a

standard insecticide (Davies *et al*., 2012b). Also, the government of Ghana is reported to have spent several millions of Cedis in the disinfestation process across the country (Deku *et al*., 2021). The process of developing and marketing a new active insecticidal agent is costly with an estimated cost of over US$ 180 million (Doggett *et al*., 2012b).

2.3 Clinical signs and symptoms of bedbug bites

Bed bug infestations come with unbearable consequences. Among them include bites on the arms and legs. However, these bites can occur on any exposed part of the skin; they normally appear as erythematous papules with a central punctum and 2-5mm pruritic in either linear, sequential, or clustered arrangements in a "breakfast, lunch and dinner pattern" (Lovgren & Darling, 2015). Although they may appear painless, the bites might cause severe reactions ranging from asymptomatic to itchy, swollen, and blistered, often causing secondary bacterial infections (Goddard *et al*., 2015). These bites have repeatedly been misdiagnosed as mosquito bites, scabies, spider bites, chickenpox, hives, antibiotic reactions, staphylococcus infections, and food allergies (Balster, 2011).

Furthermore, the pest has been reported to take a large blood meal of about 13.2ml with a possibility of resulting in anemia during high infestation instances (Trudnowski & Rico, 1974). Those infested usually have different reactions to the bedbug's saliva, where approximately 70% have been linked to related allergic effects such as urticaria, itchmen erythematous, and papular lesions (Rahim *et al*., 2016). Also, secondary bacterial infections such as lymphangitis, ecthyma, impetigo, and cellulitis (Koganemaru & Miller, 2013) have as well been reported. In some instances, the infestations have entirely compromised people's lives. This has resulted in compromised mental health due to agitation, paranoia, emotional distress, insomnia, depression, and even suicidal thoughts (Goddard & De Shazo, 2012). There has been high level of stigma and social isolation among the victims since the pest is associated with poor housekeeping and hygiene. A long-term psychological trauma out of the bites can lead to a delusionary state to the patient. The nuisance

nature of this indoor pest has since been compared to rats which have been classified as more socially acceptable than the bedbugs (Doggett *et al*., 2012b).

2.4 Biology of bedbugs

2.4.1 Taxonomy

Bedbugs are obligatory hematophagous ectoparasites belonging to the family Cimicidae within the order Hemiptera (Tiara Dewi, Muhammad Amir Masruhim, 2016). They belong to kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Hemiptera, Family: Cimicidae, Genus: Cimex and several species which include; *C. adjunctus, C. antennatus, C. brevis, C. columbarius, C. emarginatus, C. incrassatus, C. japonicus, C. latipennis, C. pipistrelli, C. lactularius, C. insuetus, C. himalayanus, C. flavifuscus, C. dissimilis, C. cavernicola, C. burmanus, C. hemipterus* and *C. pilosellus* among others.

Among the 90 or so species of family Cimicidae, the common bedbug (*Cimex lectularius*) and the tropical bedbug (*Cimex hemipterus*) have been identified to entirely depend on human bloodmeal (Doggett *et al*., 2012b). The two are very similar and prevalent in temperate, tropical and, subtropical regions (*C. lectularius* 28–29˚, *C. hemipterus* 32– 33˚C) (Koganemaru & Miller, 2013); but, both species can be found beyond their normal ranges (Gbakima *et al*., 2002). In previous studies, the two common species have been found to coexist in some regions of the world, such as Japan, Taiwan, Australia, Florida, Africa (Mohammad *et al*., 2020), Brazil (Zorrilla-Vaca *et al*., 2015), Asia and Middle East (Society, 2017; Zhang *et al*., 2021).

2.4.2 Anatomy

The anatomy of bedbugs can be classified into internal and external anatomy. The internal anatomy of the males is that they mainly have scent glands, testis and intestines. The females on the other hand have scent glands, intestines, genital chamber, mycetome, ovaries and ovarioles. The external anatomy of bedbugs comprises of three body parts (head, thorax and abdomen), two antennae, two eyes,

six legs, reddish brown in color and bright red when engorged after a bloodmeal. Further, they have piercing sucking mouth parts known as proboscis. The differences in their sexes are that the males have sharpened genitalia while the females have a softened abdominal region meant for copulation (Ndiaye *et al*., 2022). Other household pests such as immature cockroaches, beetles, spiders, and fleas have often been confused with bedbugs (Wu *et al*., 2014a).

Generally, the most common African bed bug is characterized by being flat, broad oval, dark brown in color, visible to the naked eye and an ability to travel averagely less than 2 minutes the length of a hospital bed. Adult bedbugs are reddish brown, approximately 5 mm, and dorsoventrally flattened, while their nymphs are approximately 1-4 mm in length, translucent and yellowish-white in color (Koganemaru & Miller, 2013). The pests are normally cryptic, photophobic, and thigmotactic with a typically buggy, sickly sweet odour (Doggett *et al*., 2012b). In addition to the bulky smell, the presence of faecal spots, empty egg cases, eggs, nymphs, shed exoskeletons, and dead or live bedbugs are quick signs of an emerging infestation (How & Lee, 2010).

2.4.3 Life cycle and feeding habits

It is interesting how bedbug mate since they are the only hematophagous arthropods that experience traumatic insemination. The male usually pierces a hole using the paramere into a specific area of the female skin (mesospermalege) and pump sperms and seminal proteins through the wound. The female then evolves an elastic, resilin-related biomaterial at the site of traumatic insemination. Reproduction occurs by the female bugs producing an average of 200-500 eggs in their lifetime which hatch in two weeks (Delaunay *et al*., 2011). They then undergo a five nymph stages in 5-6 weeks for them to mature in a life expectancy of 4 to 6 months (Doggett *et al*., 2012c). Their life cycle is paurometabolous, meaning that the young ones are similar to the adults and all life stages are hematophagous.

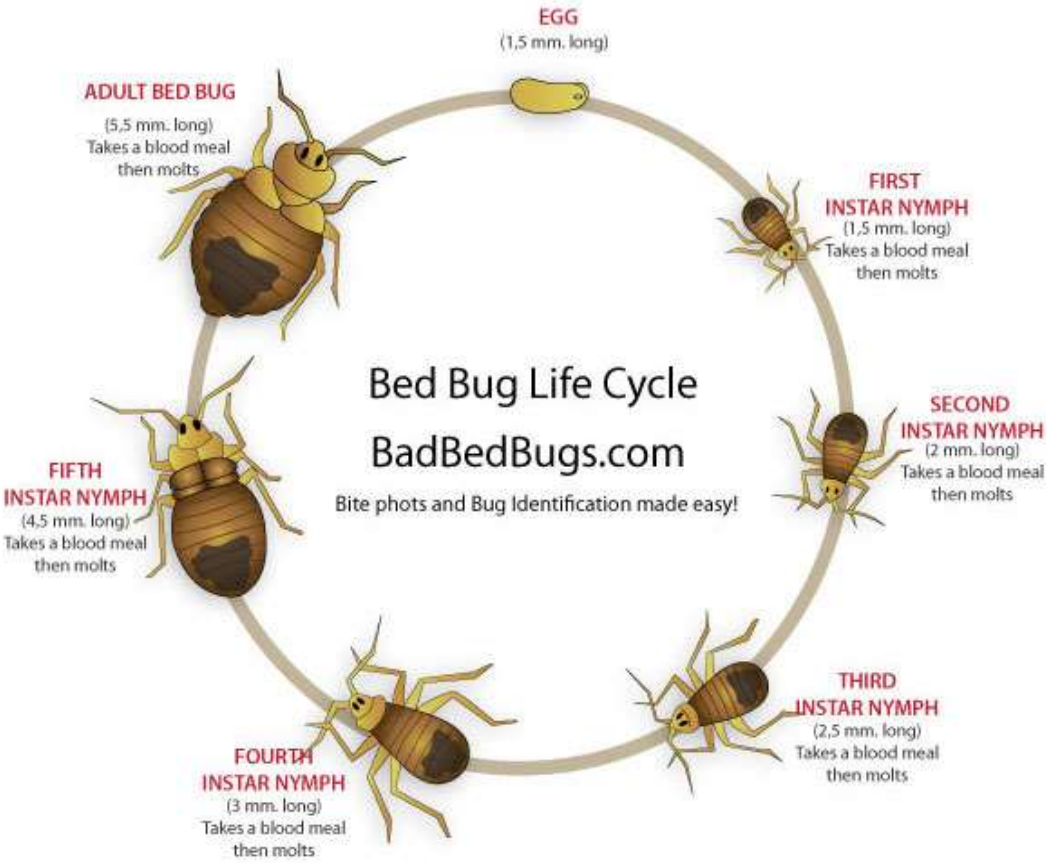


Figure 2.1: Life cycle of Bed bugs Source: https://www.bing.com (2011).

2.4.4 Gut microbiome composition

A bed bug's midgut microbiome/microbiota simply represents the collective genome of the micro-organisms residing in that particular environmental niche or the micro-organisms themselves respectively. The gut microbiota is a major factor that determines pathogen and infection transmission. Insect bodies harbours several diverse bacterial communities ranging from commensal, parasitic, to facultative or obligatory mutualistic associations with their hosts, inhabiting majorly the gut (Douglas, 2015; Feldhaar, 2011; Komaki & Ishikawa, 2000). The gut microbiome is essential in nutrition, development, protection, reproduction, insecticide resistance and growth (Dillon & Dillon, 2004; Engel & Moran, 2013; Lim & Ab Majid, 2021). Therefore, characterizing the core constituents of microbial communities associated

with arthropods is of crucial importance as a primary step for elucidating the nature of host/microbe interaction and the potential of the insect vectoring important human or agricultural pathogens (Meriweather *et al*., 2013).

The first endosymbiont microbial species in bedbugs were identified through microscopy in 1921 (Meriweather *et al*., 2013). Further, past studies reported for example, bedbugs harbours *Wolbachia*, a hereditary, gram negative, intracellular alphaproteobacterial which is a transovarially transmitted endosymbiont among other bacterial (Fisher *et al*., 2018; Kakumanu et al., 2020). Hematophagous pest arthropods including cimicidae entirely depend on vertebrates' blood for growth and development (Rio *et al*., 2016; Soh & Veera Singham, 2022). Nonetheless, their bloodmeals is a limited of essential nutrients such as amino acids and vitamins which are imperative for the host survival hence the symbiotic relationship between the blood-sucking insects and the microbes for provisioning of thes lacking nutrients (Douglas, 2017; Husnik, 2018). The development and advancement of bioinformatics and next-generation sequencing (NGS) techniques such as 16S metagenomics, has facilitated the discovery of gut microbiota in various blood-sucking insects and other arthropods such as tsetse flies (Michalkova *et al*., 2014), ticks (Duron *et al*., 2018), human lice (Kirkness *et al*., 2010), mosquito (Y. Wang *et al*., 2011) and common bedbugs (Meriweather *et al*., 2013).

2.4.5 Pathogen transmission

Bedbugs have for long been hypothesized as potential disease vectors for nematodes, protozoans, bacteria and viruses with no casual evidence as at present. Additionally, they have been proved unable to transmit non-arthropod borne viral pathogens such as HIV, hepatitis B,C and E viruses (Doggett *et al*., 2012c) but they can retain HIV for approximately 8 days and HBV for at least 7.5 weeks (Harlan, 2006). However, it is worrisome arthropod borne viral pathogens such as kaeng khoi virus, buggy creek virus and morgan virus which have not been fully scientifically investigated since they experience transmission cycles involving insects hence with a high likelihood of being transmitted by bed bugs unlike with non-arthropod borne viral pathogens (Adelman *et al*., 2013).

Past studies have raised suspicion of bugs serving as vectors for almost 45 human infectious diseases such as yellow fever, tuberculosis, leprosy, filariasis, *kalaazar* (leishmaniasis), cancer, small pox, *Rickettsia parker, T. Cruiz, B. Quintana, methicillin-resistant staphylococcus aureus, Burkholderin multivorans, Vancomycin-resistant Enterococcus, Bacillus licheniformis, Peniccilium chrogenum, stenotrophomonas matophilia,, Enterobacter hormaechei, hepatitis C virus* and *staphylococcus saprophyticus* (Delaunay *et al*., 2011). Insect vectors for *Bartonella* and *Typanosoma* (human body louse and triatomine bugs, respectively) are very similar to bedbugs. This raises questions on their ability to transmit *T. cruzi* and *B. quintana* since the two can survive in bed bugs under laboratory conditions. Other members of the family Cimicidae including, swallow bugs (*Oeciacus vicarious* Horvath) are known biological vectors for arboviruses i.e. Stone Lake Virus (Brault *et al*., 2009), Fort Morgan virus (Rush *et al*., 1980), and Buggy Creek virus (C. R. Brown *et al*., 2010). If other Cimicidae families have the ability to transmit pathogens, why not bed bugs?

2.4.6 Control methods of bedbugs

2.4.6.1 Chemical control

This is the common method of bedbug control. It involves mainly the use of insecticides such as Organochloride dichloro-diphenyl trichloroethane (DDT), pyrethroids, diatomaceous earth, dichlorvos, pyrroles (chlorfenapyr) and neonicotinoids. DDT was the among the first insecticides to be used in the management of the pest during and after World War II and reported to have gained resistance around 1947 (Dang *et al*., 2017b). Pyrethroids are effective and efficient neurotoxic insecticides known to be products of synthetic analogs of pyrethrin. Today, pyrethroids are the main insecticides used in the fight to eradicate bedbugs. However, their resistance has been reported across the globe (Dang *et al*., 2014; Romero *et al*., 2007a; Tawatsin *et al*., 2011a). Diatomaceous earth is a desiccant dust that is applied in areas that bedbugs harbor or travel (Wang *et al*., 2009). Neonicotinoids class of insecticides isnclude Imidacloprid. They have been used together with pyrethroids in order to improve their efficacy though resistance has as

well been recorded (Romero & Anderson, 2016). Pyrroles are a class of pro-insecticides usually activated by cytochrome P450 monooxygenases (P450s) to a more active metabolite. Their mode of action is that they are mitochondrial electron transport inhibitors (METI) disrupting the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) in the mitochondrial cells thus causing cell dysfunction and death. Nevertheless, their resistance has been documented (Jiang *et al*., 2015; Nicastro *et al*., 2013; Perumalsamy *et al*., 2010; Silva *et al*., 2016). Other insecticides that have been useful in the management of the pest include organophosphates (e.g., malathion) and carbamates (carbaryl) (Dang *et al*., 2017b).

2.4.6.2 Non-chemical control

2.4.6.2.1 Heat

Heat exposure is a traditional control measure, whereby bed bugs die at ≥ 41ºC with exposure time reducing with increase in temperature (Pereira *et al*., 2009). This method is limited because large scale heat treatment is costly, causes damage to heat sensitive items and the inability of the lethal temperatures to reach deep into the cracks and crevices.

2.4.6.2.2 Freezing

This is achieved by placing the infested items in a freezer or using dry ice to kill the bedbugs and eggs. The freezing temperatures range at -17 ºC for 2 hours for effective elimination of the pests and their eggs. This method is easily accessible and inexpensive when dealing with small items (Fong *et al*., 2012).

2.4.6.2.3 Vacuuming

It involves exposing the infested items such as mattress, furnishings, carpets, cracks/crevices, etc. in a vacuum. A designated vacuum with high efficiency particulate air (HEPA) filters is usually recommended in order to avoid contamination from bedbug allergens. The vacuum bags are then safely bagged and sealed for disposal (Kells, 2006b).

2.4.6.2.4 Canine detection unit

This involves the use of specially trained dogs for inspection of infestations, determining the extent of infestations and monitoring bedbug treatments. This control method is expensive with high possibility of false positives since it is mainly applied in large area (Fong *et al*., 2012).

2.4.6.2.5 Disposal of infested items

Disposal of heavily infested items and furnishings that cannot be treated has been useful in the past. Nevertheless, care must be taken not to reintroduce the pest with replacement of second-hand items. Furthermore, this control method is burdensome and may propagate the spread of bedbugs elsewhere. Alternative ways to eradicate bedbugs include sealing, use of metal furniture, clutter removal, encasements, use of steam, laundering, fumigation, use of detergents and other essential oils.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Field survey

3.1.1 Study area

A survey was conducted among the residents of nine counties of Kenya including Nairobi (1.2921° S, 36.8219° E), Mombasa (3.9768° S, 39.7137° E), Machakos (1.5177° S, 37.2634° E), Makueni (2.2559° S, 37.8937° E), Kericho (0.1828° S, 35.4782° E), Bomet (0.8015° S, 35.3027° E), Narok (1.1041° S, 36.0893° E), Kiambu (1.0314° S, 36.8681° E) and Kisumu (0.0917° S, 34.7680° E) with their GPS location coordinates recorded and later used to build the predictive model. These counties represent diversity in cultural practices, livelihood strategies (such as fishing, tourism, farming), and infrastructure development. They comprise different altitudes above sea level, temperatures, and differing in average annual rainfall.

3.1.2 Survey for household's knowledge and perceptions on bedbugs

This study was a community-based cross-sectional survey conducted from November - December 2020. It was based on a stratified, systematic random sampling where 100 respondents were selected from each county. Sample size determination was based on a statistical formula described by (Fisher *et al.*, 1998).

A total number of 900 respondents were randomly selected and the household head or the representative showing willingness and consent was interviewed face-to-face. The interview was conducted using a semi-structured questionnaire prepared in the English language (Appendix I). The questionnaire was translated into the local native language (Kiswahili) to avoid biasness and improve the understanding between the enumerator and the respondent. Prior to the commencement of the survey and authentic data collection, a pre-testing exercise was performed by training enumerators on a similar socio-demographic pattern. This was useful for improving the quality of data, ensuring validity, familiarizing the enumerators with the questionnaire, and data handling.

The information collected using the semi-structured questionnaire included residents' socio-economic profiles, knowledge, and perceptions on the pest, bedbug incidence, and management practices. The socio-economic profile factors addressed in the survey comprised gender, age, education, access to basic social amenities, and household size. The study also prioritized the financial consequences, the severity of the bites, perceptions of respondents on the pest, and management practices for its control.

Survey data were checked for errors, completeness, summarized, and entered in Microsoft-Excel. It was then cleaned and transferred to Statistical Package for Social Science (SPSS) version 25 software (IBM Corp., Armonk, NY) for purposes of descriptive statistics (means and percentages). Furthermore, those who never responded to some questions were excluded from the study. In contrast, in instances where more than one reason was given for a single question, percentages were calculated based on each group of similar responses. Chi-square was performed to determine the differences regarding socio-demographic characteristics, knowledge, and perceptions on bedbugs and control practices. Additionally, data were disaggregated by gender and age categories to understand the existing differences among the various respondent categories. Besides, F-test statistics was performed on the ages of respondents to determine the mean, standard deviation and statistical significance. The level of significance was considered when the p-value was below 5%.

3.1.3 *I*nfestation dynamics model of bedbug

3.1.3.1 M**odel** simulation assumptions

Houses infestation dynamics was studied following Susceptible-Infested-Treatment (SIT) model (Kermack & Mckendrick, 1991). Therefore, houses in the community were classified into three groups: susceptible, infested or treated. Within a house, bedbug population dynamics was ignored, while it was considered where infested houses have some potential to spread the infestation to other houses in the community and a population of bedbugs in an infested house has some probability per unit of time of becoming extinct either naturally or after treatment. In the

infestation dynamics, the rate of house infestation depends on the number of infested houses as well as the proportion of treated houses in the community. It was assumed that infested houses (I) spread the infestation at the rate β and only a fraction S/N of the houses is susceptible (S) to infestation. Infested houses become extinct at a certain rate known as rate γ. Infested houses are treated at the rate τ and the protection conferred is lost at the rate α. Ordinary differential equation developed to study SIT model were used in this study (Kermack & Mckendrick, 1991). All the models used have the generic formulations displayed below:

$$\frac{dS}{dt} = \frac{\beta}{N}SI + \gamma I + \alpha T \qquad (1)$$

$$\frac{dI}{dt} = \frac{\beta}{N}SI - (\gamma + \tau)I \qquad (2)$$

$$\frac{dT}{dt} = \tau I - \alpha T \qquad (3)$$

Where $\beta > 0$, $\tau > 0$, $\alpha \geq 0$ and $\gamma > 0$. The total population size is $N = S(t) + I(t) + t(t)$. The initial conditions satisfy at $S(0) > 0$, $I(0) > 0$, $T(0) \geq 0$ and $S(0) + I(0) = N$, where N is the constant total population size, $dN/dt = 0$.

3.1.3.2 Infestation dynamics models implementation

The method used to implement the infestation dynamics model of the pest is based on the system thinking approach with its archetypes (Causal Loop Diagram (CLD), Reinforcing (R) and Balancing (B)) by a mental and holistic conceptual framework. This is important for mapping how the variables, issues, and processes influence each other in the complex interactions of bedbugs within and between houses and their impacts. Despite these archetypes being qualitative, they are necessary for elucidating and disclosing the basic feedback configurations that occur in houses and their environs when infested with pests like bedbugs. A dynamic model was generated by converting the causal loop diagram (CLD) obtained using stocks, flows,

auxiliary links, and clouds. Consequently, these in turn were translated into coupled differential equations for simulations.

The SIT model was translated into causal loop diagram where arrows show the cause-effect relations where positive sign indicates direct proportionality of cause and effect while negative sign shows inverse proportionality relations, and two different scenarios have been assessed: (i) homogeneous houses where there is a single community of houses of the same quality, and (ii) heterogenous houses where there is a community of good and bad houses. Ancient houses and filled with old or secondhand furniture are considered bad houses as they may sustain high level of bedbug infestation; and new houses don't provide well enough conditions for bedbug population to survive, and they are called in the model good houses (Gurevitz *et al*., 2011). Bad houses are considered to act as sources while good houses act as sinks, but all together are randomly distributed where each house has the same probability to contact good or bad houses.

In the scenarios of *homogeneous houses*, the causal loop diagram (Fig. 3.1) has two feedback loops: (a) one positive, as the number of infested houses increases, the probability to get susceptible houses infested also increases resulting in infested houses increase; (b) one negative, as the infested houses increases, the treated houses increase resulting in susceptible houses decrease. The causal loop diagram is displayed in Fig.3.1A while Fig.3.1B showed the stocked and flows diagram and axillary variables obtained from causal loop diagram.

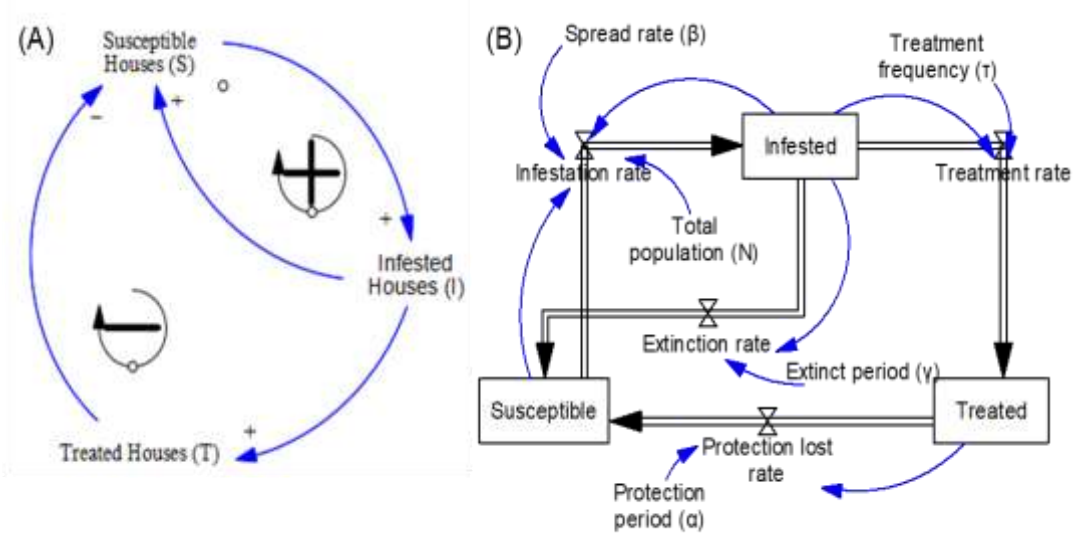


Figure 3.1: Susceptible-Infested-Treatment (SIT) model translated into causal loop diagram (A) and stock and flow diagram (B) for homogeneous houses in the community.

Susceptible, infested and treated houses are stocks in the system, representing the number of houses susceptible, infested, and treated, respectively at a given point of time. The rates represent in and out-flows of the diagram. Auxiliary and constants that drive the behavior of the system were connected using information arrows within them and flows and stocks to represent the relations among variables in terms of equations.

In the scenarios of *heterogeneous houses,* the causal loop diagram (Fig.3.2A) comes with the two previous feedback loops but for each category of house. In addition, there is a fifth feedback loop that connect bad house to good house and vice versa.

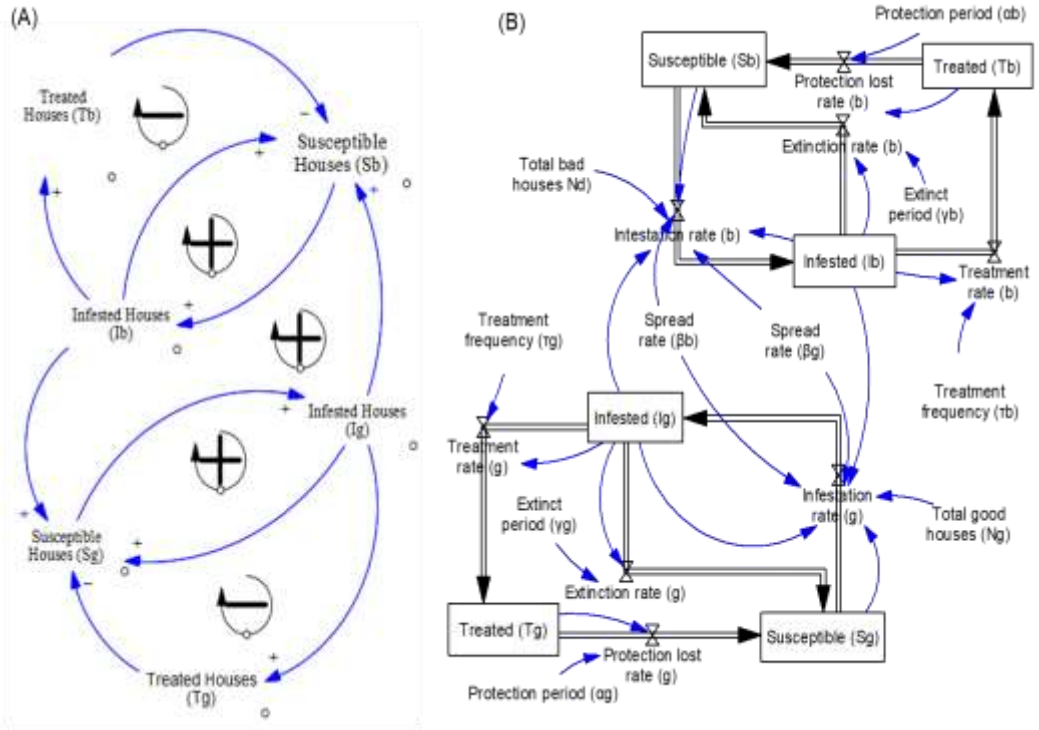


Figure 3.2: Susceptible-Infested-Treatment (SIT) model translated into causal loop diagram (A) and stock and flow diagram (B) for heterogeneous houses in the community

Therefore, as the infested bad houses increase, the probability to infest good houses increases. The more they are exposed the more they get infested. In turn, as the infested good houses increase, the chance to infest susceptible bad houses increases and the more they are exposed, the more they get infested, resulting in the increase of infested bad houses. The stocks and flows diagram of each of the two categories of houses occurred with interconnection relationships between the two categories (Fig. 3.2B).

3.1.3.3 Models' simulations

The survey data (section 3.1.1) on prevalence, knowledge, perceptions and self-reported; in addition, the respondents' reported control mechanisms and their average time of effectiveness (Appendix II) were used for model simulations. The different

control methods reported were reclassified in three control approaches: chemical control, other control methods (including exposure to direct sunlight, use of hot water, painting, application of diesel, paraffin and wood ash, use of Aloe Vera extract and Herbs), and combination of chemical and other control methods. All the models' commodities and units were checked before performing the simulations. Simulation and implementation of the models were done using Vensim PLP 8.1 platform (Ventana systems, Harvard, USA). It consists of a graphical environment that usually permits drawing of Causal Loop Diagram (CLD), stocks, flow diagrams and to carry out simulations. After simulating the infestation dynamics under the two scenarios, the effect of the different control methods was explored.

3.1.4. Spatial distribution analysis of bedbugs using MaxEnt model

3.1.4.1 Environmental data for MaxEnt

The environmental variables used as the other maxent input were obtained by deriving bioclimatic, land cover, and elevation data. Bioclimatic variables and elevation (Digital Elevation Model; DEM) data were obtained from the Global Climate Data official website, Worldclim (http://www.worldclim.org/bioclim.htm) (Hijmans *et al*., 2005) including 19 bioclimatic variables (Appendix III). The land cover data were downloaded from the Global Land Cover Facility (GLCF).

In order to reduce collinearity between predictors, a collinearity test was performed on all the variables by filtering them according to the following steps (Wang *et al*., 2020): firstly, the MaxEnt model was run using the distribution data of bedbugs and 19 bioclimatic variables to obtain the percent contribution of each variable to the preliminary prediction results. Secondly, following the generation of the percentage contribution of all the variables, then imported all distribution points in Arc-GIS and extracted the attribute values of the 19 variables. Furthermore, the "virtual species" package (Leroy *et al*., 2016) in R-software was used to explore the extracted variables' clusters spatial correlation using Pearson's correlation coefficient and the cluster tree (Fig. 3.3). Thus, the final number of predictor variables after screening was 5 establishing the potential geographical distribution of bedbug, which includes Temperature Seasonality (bio4), Precipitation of Driest Month (bio14), Temperature

Annual Range (bio7), Precipitation of Driest Quarter (bio17) and Precipitation of Warmest Quarter (bio18). The land cover was considered because studies have shown its importance on insect spatial distribution (Labou *et al*., 2017; MacFadyen *et al*., 2018; Ramos *et al*., 2019) and it was setled as a categorical variable (Mudereri *et al*., 2021). Elevation was selected as variable because it greatly influences species' occurrence and dispersal by affecting the temperature, precipitation, vegetation, and sun characteristics (direction, intensity, etc.) on the earth's surface (Azrag *et al*., 2018; Bradshaw *et al*., 2019; Ramos *et al*., 2018). The study variables had different resolutions and were therefore, resampled to 1km. The variables were clipped to Kenya and Africa boundaries and converted to ASCII (Stands for "American Standard Code for Information Interchange") format using the 'raster' package (Leroy *et al*., 2016) in R statistical software (R Foundation for Statistical Computing, Viesnna, Australia).

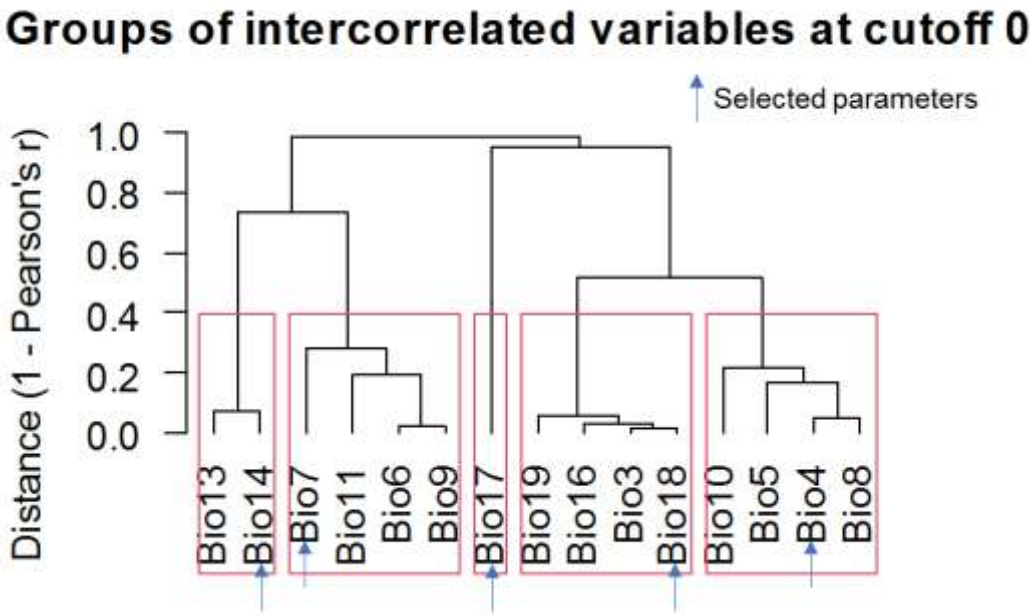


Figure 3.3: Key model predictor variables.

3.1.4.2. Distribution modelling in Kenya and Africa

In this study, a maximum entropy distribution modelling method was used. This is because it has been recommended to have the ability to perform best and remain

effective despite the use of small sample size relative to the other modelling methods (Kumar & Stohlgren, 2009).

The selected bioclimatic variables (5) and occurrence/prevalence data for bedbugs were then imported into MaxEnt model and the options of ‘Create response curves’ and ‘Do jackknife’ were selected to measure variable importance’ options. The model output file was selected as ‘Logistic’, the commonly used approach is the random portioning of distribution datasets into ‘training’, and ‘test’ sets (Fielding & Bell, 1997; Kumar & Stohlgren, 2009). MaxEnt model was run with a total number of 5,000 iterations and five replicates for better convergence of the model and rescaled within the range of 0-1,000 suitability scores using 'raster' package (Leroy *et al*., 2016) in R statistical software (R Foundation for Statistical Computing, Vienna, Australia).

The modelling performance/MaxEnt accuracy was evaluated by choosing the area under the receiver operating characteristics (ROC) curve (AUC) as the estimation index. This was important for the calibration and validation of the robustness of MaxEnt model evaluation. Furthermore, the area under the ROC curve (AUC) was necessary as an additional precision analysis (K. Zhang *et al*., 2018). The range of AUC values greater than 0.7 was considered a fair model performance, while those greater than 0.9 indicated that the model was considered an excellent model performance. Therefore, by considering the AUC values, the excellently performing model was selected to analyze the suitability of bedbugs in Kenya and Africa (Phillips *et al*., 2006; Xu *et al*., 2019; Zhang *et al*., 2018; Zhang *et al*., 2018).

The ASCII format output was then imported into QGIS 10.2, following its conversion into a raster format file using R software. This was useful for the classification and visualization of the distribution area (López-martínez *et al*., 2016; Montemayor *et al*., 2014). The potential suitable distribution of bedbugs was extracted using the Kenyan and African maps. At the same time, Jenks' natural breaks were also used to reclassify and classify the suitability into five categories, namely: unsuitable ($P < 0.2$), marginal ($0.2 < P < 0.4$), suitable ($0.4 < P < 0.6$), optimal ($0.6 < P < 0.8$) and highly suitable ($P > 0.8$) area (R. Wang *et al*., 2020).

Therefore, MaxEnt modelling was used to predict the distribution of *Cimex spp.* (Hemiptera: *Cimicidae*) in Kenya and Africa using our collected geo-referenced occurrence records.

3.2 Diversity

3.2.1 Sample collection

Live bed bug specimens were disturbed and flushed out from their hiding places (wall crevices, cracks, bed frames, cup boards, and mattresses) using a pair of forceps. A minimum distance of 10 km between the sample collection points was maintained during the whole process. Once collected, bed bugs were preserved in absolute ethanol in 50 ml falcon tubes (well-marked indicating each reference collection point), then transported to the *icipe* Arthropod Pathology Unit and stored at -20°C awaiting molecular analysis.

3.2.2 Samples identification using morphological identification

The key morphological features used in identifying bedbugs include: 1) the head has a labrum that appears as a free sclerite at the extreme anterior margin, ecdysial lines form a broad V, eyes project from the sides composed of several facets and the antennae are 4-segmented, 2) thorax is subdivided into prothorax, mesothorax and metathorax, 3) legs have all other normal parts except pulvilli and arolia, tarsus is 3-segmented with 2 simple claws, 4) the abdomen has 11 more-or-less segmented recognizable segments, 7 pairs of spiracles borne on the second to eighth segments, hosts the genital structures, paramere in males and mesospermalege in females (Usinger, 1966). Bedbug specimen morphological features were examined using Leica EZ24 HD dissecting microscope (Leica Microsystems, UK) and photos documented using the associated software.

3.2.3 DNA Extraction

Individual samples (five per household) were surface sterilized in 3% sodium hypochlorite for 30 sec, rinsed in 70% of ethanol for 30 sec, rinsed thrice in sterile distilled water then placed into 1.5 ml microfuge tubes (Eppendorf) from where

genomic DNA extraction was done using the Isolate II Genomic DNA extraction kit (Bioline, London, UK) following the manufacturer's instructions.

3.2.3 PCR Amplification and DNA Sequencing

For molecular identification of the bedbugs, mitochondria Cytochrome c Oxidase I (CO1) fragment was amplified using VF1d-t1 (5'-TGTAAAACGACGGCCAGTTCTCAACCACAARGAYATYGG-3') and VR1d-t1 (5'-CAGGAAACAGCTATGACTAGACTAGACTTCTGGGTGGCCRAARAAYCA-3') (Ivanova *et al*., 2007) primers. Polymerase chain reaction (PCR) was carried out in a total volume of 10 µl containing 5.375 µl PCR water, 2 µl buffer, 0.5 µl of each primer, 0.125 µl of *Taq* DNA polymerase, 0.5 µl of MgCL and 1 µl of the DNA template. Initial denaturation was done for 2 min at 95 ºC, followed by 40 cycles of denaturation at 95 ºC for 30 sec, annealing at 53.4 ºC for 45 sec, and primer extension at 72 ºC for 1 min, followed by a final extension at 72 ºC for 10 min. Successfully amplified products were purified using the Isolate II PCR and Gel Clean up kit (Bioline) following the manufacturer's protocol. The purified products were bi-directionally Sanger sequenced at Macrogen Europe Laboratories (Macrogen Inc., the Netherlands).

3.2.4 Genetic Diversity and Phylogenetic analysis

DNA sequences were aligned with Multiple Sequence Comparison by Log-Expectation (MUSCLE) following a quality check using Geneious v8.1.8 software (http://www.geneious.com) (Banerjee *et al*., 2017) for population genetics analysis. An additional 31 mitochondrial COI sequences were retrieved from the GenBank originating from different world regions, including Europe, India, Senegal, Malaysia, Singapore, Norway, and Kenya. Together with the ones from this study, these sequences were aligned and trimmed to 486 bp length since this region was commonly present in all 67 sequences. The remaining sequences that did not meet the 486 bp region were exempted from further analysis. A phylogenetic tree was constructed using the Maximum Likelihood (ML) method applying Kimura 2-parameter model. The reliability of the inferred tree topologies was tested by 1000

bootstrap replicates. *Triatoma dimidiata,* GenBank accession number JQ575031.1, was used as the outgroup taxon because it belongs to a different family from that of bedbugs (Hemiptera: Reduviidae). Further, pairwise genetic distance among the various haplotypes was estimated in MEGA v.6.0 using Kimura 2-parameter model.

3.2.5 DNA polymorphism and population structure analysis

This study estimated sequence polymorphism using DnaSP version 6.12.03 (Rozas *et al*., 2017). This was meant for determining descriptive statistics such as nucleotide diversity (π), number of haplotypes (H), haplotype diversity (Hd), genetic neutrality tests and mismatch distribution analysis.

Population structure was studied by performing Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) in Arlequin v3.5.2.2. Arlequin was utilized to compute Fst values (Venkatesan *et al*., 2007). The percentage of observed variance within and between groups was calculated for determining the genetic molecular variations. Significance was obtained by 1000 permutations tests.

3.3 Screening for resistance gene

3.3.1 PCR Amplification, DNA Sequencing and analysis

DNA extracted from individual bedbug samples (section 3.2.3) was used to screen for insecticide resistance. The sense and anti-sense primers BBparaF1 (5-AACCTGGATATACATGCCTTCAAGG-3) and BBparaR1 (5-TGATGGGGAGATTTTGCCACTGATG-3) were used (Zhu *et al*., 2010). PCR reaction was done using the My Taq PCR kit (Bioline, London, UK) after series of gradient PCRs to select the most optimal cycling conditions. All PCR runs were performed using the master cycler nexus gradient (Eppendorf, Hamburg, Germany). BBParaF1/BBParaR1 was used to generate fragment I containing amino acid V419L. PCRs were conducted in a total volume of 10 µl with conditions for initial denaturation as 95 ºC for 2 min, followed by 40 cycles of denaturation at 95 ºC for 30 sec, annealing at 60.3 ºC for 45 sec, extension at 72 ºC for 1 min and a final extension step at 72 ºC for 10 mins. Fragments were visualised on 2% agarose

electrophoresis gels (1× TBE). Successfully amplified products were purified using Isolate II PCR and Gel Clean up kit (Bioline) following the manufacture's protocol. The purified products were outsourced for bi-directional Sanger sequencing Inc. (Macrogen Inc., Netherlands).

The sequences were cleaned and the quality checked using Geneious v8.1.8 software. The chromatograms were visualized and edited with Geneious v8.1.8 software (http://www.geneious.com) (Banerjee *et al*., 2017). Authenticity of the sequences was determined through BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi), in the NCBI GenBank database, considering an identity percentage greater than 99%. Multiple alignment of the edited sequences was performed with MUSCLE software. The frequency of resistance was determined by considering the total number of samples that successfully amplified and sequenced.

3.4 Gut microbiome

3.4.1 MinION sequencing

The total genomic DNA of two replicates from each of the sampled areas was subjected to MinION sequencing. The MinION $1D^2$ sequencing chemistry (Oxford Nanopore Technologies, Inc., Oxford, UK) is a next-generation sequencing (NGS) technique capable of producing long reads (10,000–100,000 bp) hence resulting to longer alignments, better taxonomic resolution and higher specificity (B. L. Brown *et al*., 2017). Each of the DNA replicates was sequenced as separate samples on an Oxford Nanopore Technologies (ONT) MinION device using R9.4 flow cells to obtain the full-length 16S rRNA metagenome (~1,500 bp). Preparation of libraries was performed using SQK-16S024 16S Barcoding kit as per the ONT "16S Barcoding Kit (SQK-16S024)" protocol, following manufacturer's instructions. PCR for library preparation was carried out in a total volume of 50 µl containing 5X My Taq reaction buffer (5 mM dNTPs, 15 mM MgCl2, stabilizer and enhancer) (Bioline), 0.5 pmol µl -1 of each 16S barcode, 0.0625 U µl -1 MyTaq DNA polymerase (Bioline), and 10 ng µl -1 of DNA template. The thermal cycling conditions in a Mastercycler Nexus gradient thermal cycler (Eppendorf, Germany), were as follows: Initial denaturation was done for 2 mins at 95°C, followed by 35

cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 40 sec, and extension at 72°C for 1 min followed by a final extension at 72°C for 10 mins. Finally, prepared libraries were purified, pooled and diluted to 4 nM prior to sequencing on MinION sequencer. The runs ranged from 4 to 8 hours.

3.4.2 Base calling and read preparation

The generated MinION reads were live base called using the ONT Albacore in the MinKNOW software (v19.05.0). Consequently, all resulting raw datasets were deposited in the NCBI database following a quality assessment via MinKNOW. The raw reads from MinION were then uploaded to the ONT cloud for downstream analyses via EPI2ME (v2.59.1896509) which followed an immediate return of the base-called data to the host computer still in the form of FASTQ files. "What's in my Pot" (WIMP) (Juul *et al*., 2015), is a workflow that classifies and identifies species in real-time. The process of extracting, characterizing the numbers of reads and assigning taxonomy was performed using FASTQ-WIMP (v3.2.1) (Hocquellet *et al*., 1999) (https://epi2me.nanoporetech.com/). Further, FASTQ-WIMP, which is reported to work for long-read data (Irinyi *et al*., 2019), was used to perform analysis and assigning the datasets taxonomic identification by comparing the reads against a NCBI database for bacteria. WIMP makes use of Centrifuge software (Kim *et al*., 2016). This software facilitates accurate identification of reads when using databases containing multiple highly similar reference genomes, such as different strains of bacterial species.

3.4.3 Bacterial diversity statistics

Direct quantitative comparison of abundances was performed at genus level by constructing stacked bar plots. This was important for viewing the cumulative reads from the samples for each of the species, county and country level. Only Taxa that reached above 0.5% cut-off was used in stacked bar plots, while the rest (below 0.5%) were collapsed into "Others" category. Merging minor taxa is recommended when the number of bacterial species is large and diversified since it helps better visualization of significant taxonomic patterns, especially at higher-levels (e.g., genus) as compared to lower-levels (such as species). Alpha diversity analysis

(evenness, richness and the Shannon-Wiener index) were used to determine the bacterial diversity of each sample. Beta diversity was computed using the Bray Curtis dissimilarity index (Beals, 1984; Patel, 2019) to determine the diversity of bacterial genera among the samples. Inter-population distances were visualized using principal coordinate analyses (PCoA) computed in R software v3.5.3, using the 'vegan' package (Oksanen *et al*., 2007).

CHAPTER FOUR

RESULTS

4.1 Field survey

4.1.1 Morphological identification of the bedbugs

Samples bore the defined characteristics of bedbug's that belong to the genus Cimex. The adult bedbugs were wingless, dorsoventrally flattened, and brown in appearance (Fig. 4.1, A and B). The pronotum (Fig. 4.1, D) was expanded with fine hairs that were shorter than the width of the eye. The labrum (Fig. 4.1, D) was at the extreme anterior margin of the head, had 4-segmented antenna (Fig. 4.1, C), had less than 11 recognizable segments on the abdomen (Fig. 4.1, B) and a proboscis length that reached the middle of the first coxa (Fig 4.1, C).

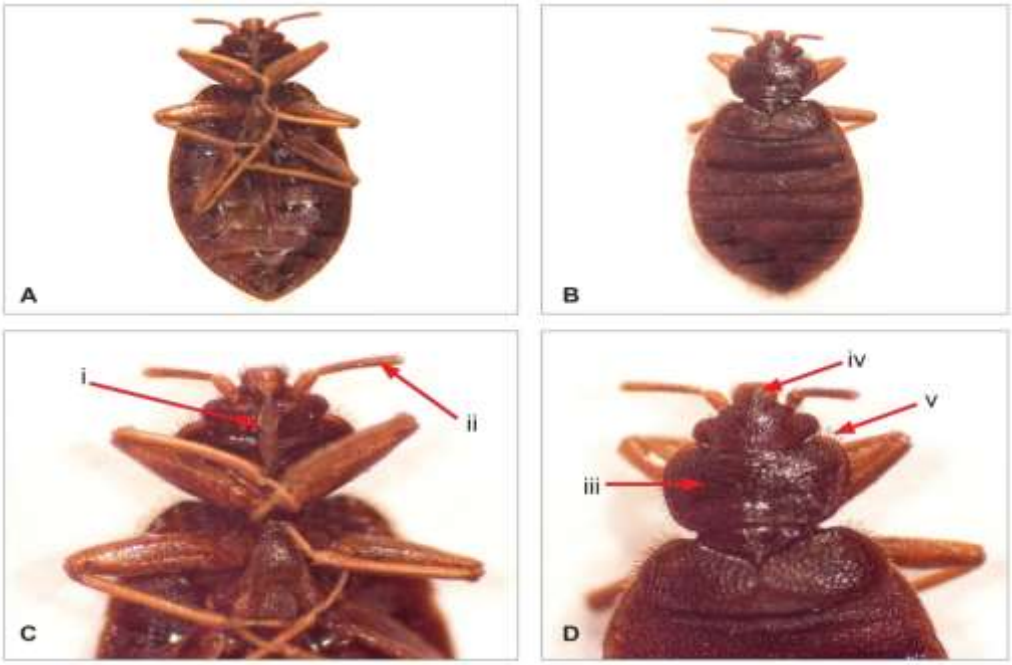


Figure 4.1: Morphological features of bedbug: (a) ventral side, (b) dorsal side, (c) ventral head section; (i) proboscis; (ii) antenna, (d) dorsal thoracic section; (iii) pronotum; (iv) Labrum; (v) fine hairs.

4.1.2 Socio-demographic profile of the respondents

The results showed that 49.8% of the respondents were females compared to 50.2% males. The minimum and maximum ages were 18 and 96 years, respectively, with a mean age of 38.15 years. Overall, 84.2% of the households consisted of less than three occupants while 15.7% had three occupants and above (Fig. 4.2). This was based on a principle that one must have lived in the household for at least three months prior to the interview. About 58.9% of the respondents indicated that they had access to basic amenities in < 30 minutes, while the rest, 41.1% did not (Fig. 4.2).

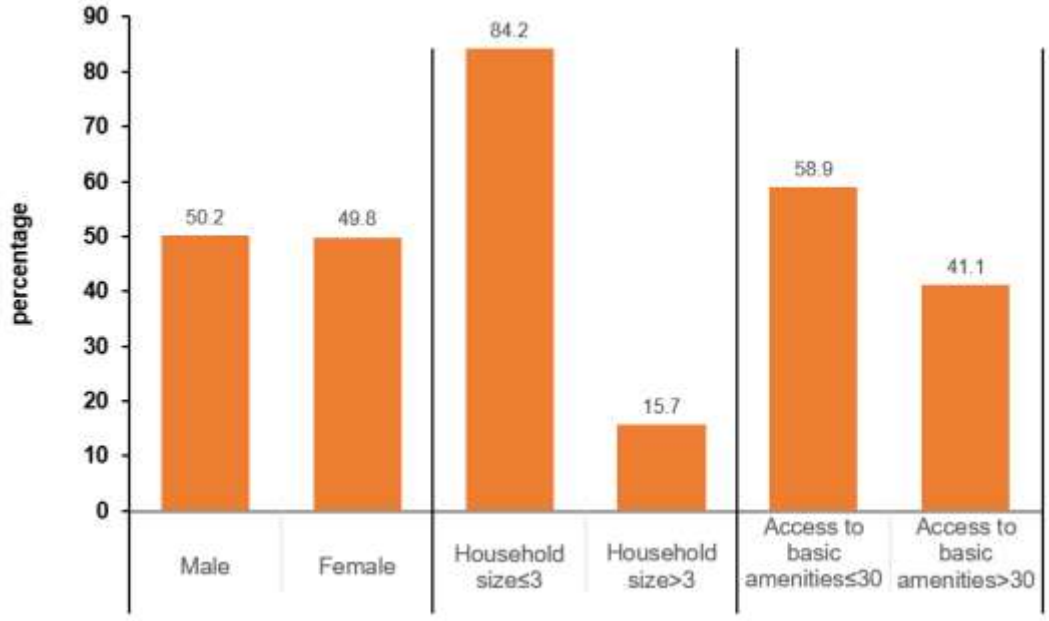


Figure 4.2: The socio-demographic profile of the respondents

4.1.3 Bedbug perceptions and incidence in the communities

Most of the respondents could identify bedbugs from other known ectoparasitic arachnids like ticks, fleas, mites, and jiggers. About 80% of the respondents indicated bedbug infestation as a weird scenario, unlike the rest (20%) who perceived it as a normal occurrence in the society. In addition, more than 97% of the respondents affirmed that the pest is most active at night. When asked to indicate the severity of the bites, most respondents (68.9%) reported them to be mild, 28.8% said

they were severe while 2.3% considered the bites side effects free. Those who experienced severe bites had to seek medical attention either by over-the-counter medication or at a medical facility. It was reported that among the recommended and received medication, anti-allergies, painkillers, and anti-inflammatory drugs were the commonest (Appendix IV). Out of the 900 respondents, 89.8% reported having disinfested their households more than once compared to 10.2% who did it once a year. Besides, bedroom/mattresses (34%), furniture (28%), cracks/crevices (24%), and clothes (14%) were the most infested household areas.

4.1.4 Bedbug management practices

The residents reported having applied various bedbug control methods. These were generally grouped as; chemical and cultural, chemical only, cultural only, and botanical methods. Most respondents (79.3%), confirmed to have used both chemical and cultural practices, followed by cultural only 10.3%, chemical only 9.2% and botanical 1.1% (Fig. 4.3A). Besides, the applied cultural practices included use of hot water, exposure to sunlight, paraffin, diesel, and detergents while botanicals comprised the use of aloe vera extract and herbs (waragi). There was a statistically significant difference between the various control methods across the sampled counties (X^2 = 145.226; df = 24, P ≤ 0.000) (Fig. 4.3A). However, when asked about their effectiveness, the use of chemical methods (pesticides) dominated with 57.9% followed by cultural methods at 30%, combined use of chemical and cultural methods at 6.1%, and use of botanicals was the least with 2.4%. Furthermore, only 3.6% of the respondents reported that none of the control strategies was effective enough (Fig. 4.3B). A significant statistical difference was detected in the level of effectiveness between different control practices across the surveyed counties (X^2 = 495.555; df = 32, P ≤ 0.000). A total of 21 pesticides belonging to different classes of WHO classification were reported, where the most commonly/frequently used across the counties contain chlorpyrifos, carbaryl, cipronil, pyrethrin, imidacloprid, and gichlorvos as the active compounds (Appendix V). Across gender and age categories, choice and preference of the control strategies was statistically insignificant (X^2 = 2.506; df = 3, P ≤ 0.04 and X^2 = 11.109; df = 12, P ≤ 0.05 respectively).

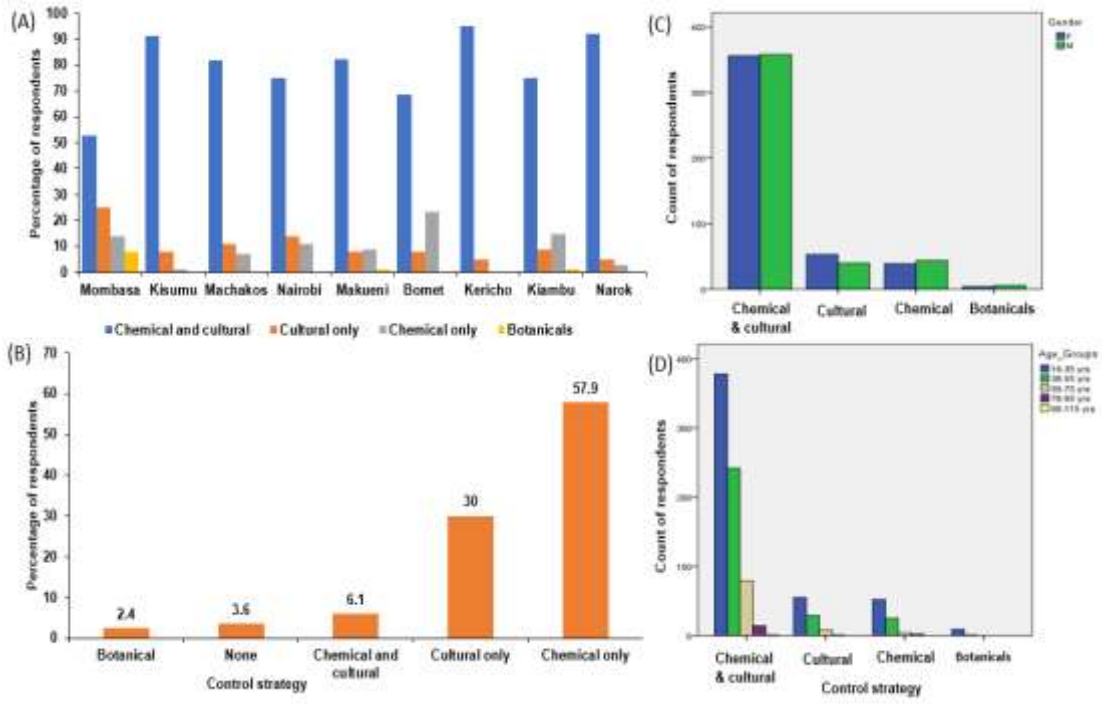


Figure 4.3: Control strategies recorded across different counties (A), the effectiveness of control and management practices (B), perception based on gender (C) and age group (D).

Male respondents were reported to have been more likely to use combined chemical and cultural methods, chemical only, and botanical control methods than their female counterparts who preferred cultural management practices. Chi-square analysis exhibited that there was no significant difference between respondents in terms of gender (X^2 = 2.506; *df* = 3, P ≤ 0.474) and age category (X^2 = 11.109; *df* = 12, P ≤ 0.520) as regard to the choice control strategy (Fig. 4.3C and D).

4.1.5 Infestation dynamics of bedbugs in residential communities

The outcomes of the model showed that the infestation marginally increased while the number of susceptible houses decreased at the same rate and the equilibrium was reached after 4 months (Fig. 4.4A). Both dynamic curves reached a plateau after 10 months of simulation where about 94% of houses in the community were infested in the scenarios of homogeneous houses. When houses were categorized into bad and good houses, the infestation in the community increased with a smooth slope and

reached the plateau with some delay showing 15 months of simulation. Furthermore, the level of infestation for good houses (Ig) was much lower as compared to that of infested bad houses (Ib), translating into 42% and 58% of infestation, respectively (Fig. 4.4B). The sensitivity test showed that bedbug infestation among the community is highly influenced by the level of mobility of individuals in the population (Fig. 4.4C). For instance, the infection rate tends to increase when mobility level is high (Fig. 4.4C).

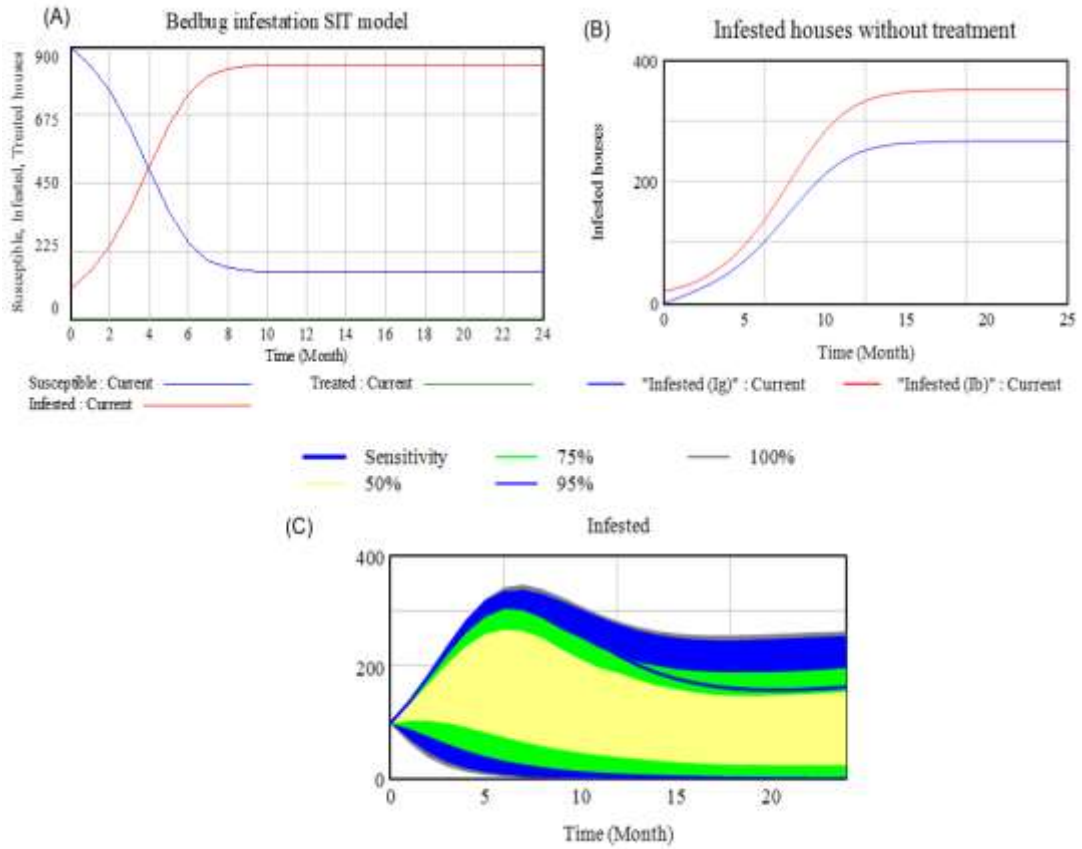


Figure 4.4: Infestation dynamic in homogeneous houses (A), in heterogeneous houses (B) communities and sensitivity test of the model (C).

4.1.6 Control strategies performance

A simulation considering the use of chemical control method (insecticides) showed that the number of susceptible houses decreased to approximately 300 in the first eight months (Fig. 4.5A). Also, the peak of infested houses was reached after seven

months, translating to around 450 houses. Further, the peak of treated houses was reached after 14 months, translating to approximately 400 houses (Fig. 4.5A). Additionally, our SIT model was further simulated to understand the effects on using other control methods. The number of susceptible houses decreased drastically to around 225 houses in nine months while that of infested houses increased to approximately 500 houses in a period of eight months (Fig. 4.5B). The peak of treated houses was reached after 12 months translating to around 300 houses as well (Fig. 4.5B). When combining chemical method and other control methods, it was noted that an equilibrium involving susceptible, infested, and treated houses was reached after eight months translating to around 300 houses (Fig. 4.5C). Additionally, the peak of infested houses was reached after six months translating to approximately 300 houses, while that of the treated house was reached after 12 months translating to around 450 houses (Fig. 4.5C). It was noted that the use of other control methods for disinfestation was less effective as compared to the other scenarios (Fig. 4.5B). In the scenarios of heterogenous houses within the community, focusing on the treatment of only bad houses with treatment rate of $\boldsymbol{\tau}_b = 0.1$ gave a better result in reducing the number of infested houses than treating both good and bad houses with $\boldsymbol{\tau}_g = 0.06$ $\boldsymbol{\tau}_b = 0.085$, respectively (Fig. 4.5D).

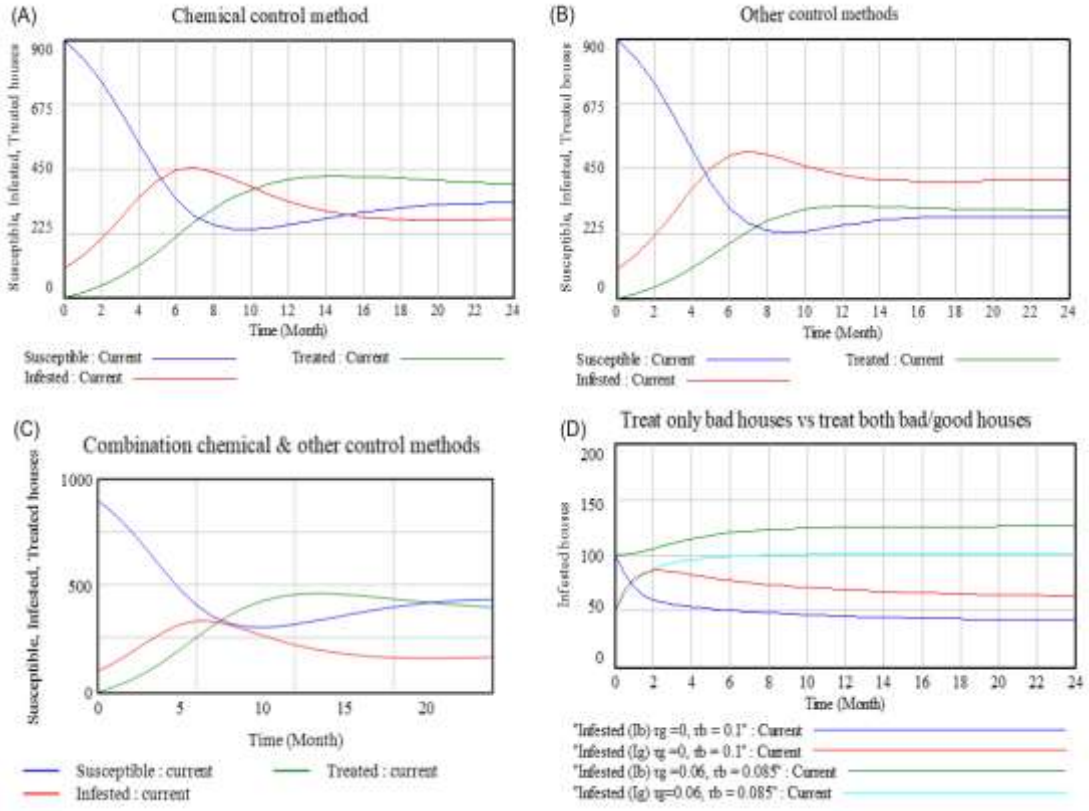


Figure 4.5: Control methods performance and management strategies

4.1.7 Potential distribution of bedbugs in Kenya and in Africa

The output map of Kenya showed regions with optimal to suitable for bedbug occurrence in the whole country (Fig. 4.6A). No region was unsuitable found for its occurrence in Kenya. The Eastern and central regions of the country were more optimal while other regions varied from marginal to suitable zones and highly suitable zones could be found here and there across the country (Fig. 4.6A). Similar results were found at continental level varying from marginal to optimal occurrence zones across Africa (Fig. 4.6B). Eastern and central African countries were more optimal with highly suitable zones in southern countries while it can be found here and there in West-African countries.

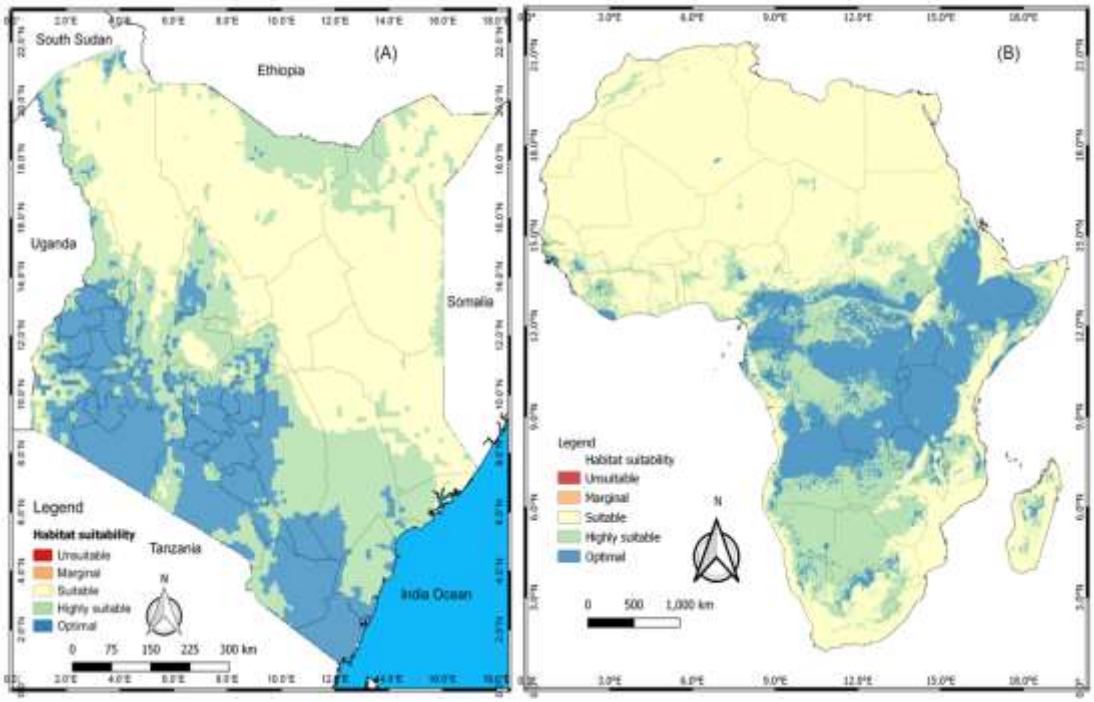


Figure 4.6: Potential geographical distribution of bedbug in Kenya and in Africa

4.1.8 Bedbug Cimex spp. habitat suitability model

The AUC values were all higher than 0.6, indicating the optimal occurrence area of the pest (Fig. 4.7A and B). This shows that the model successfully predicts the suitable habitat of the bedbug in Kenya and in Africa.

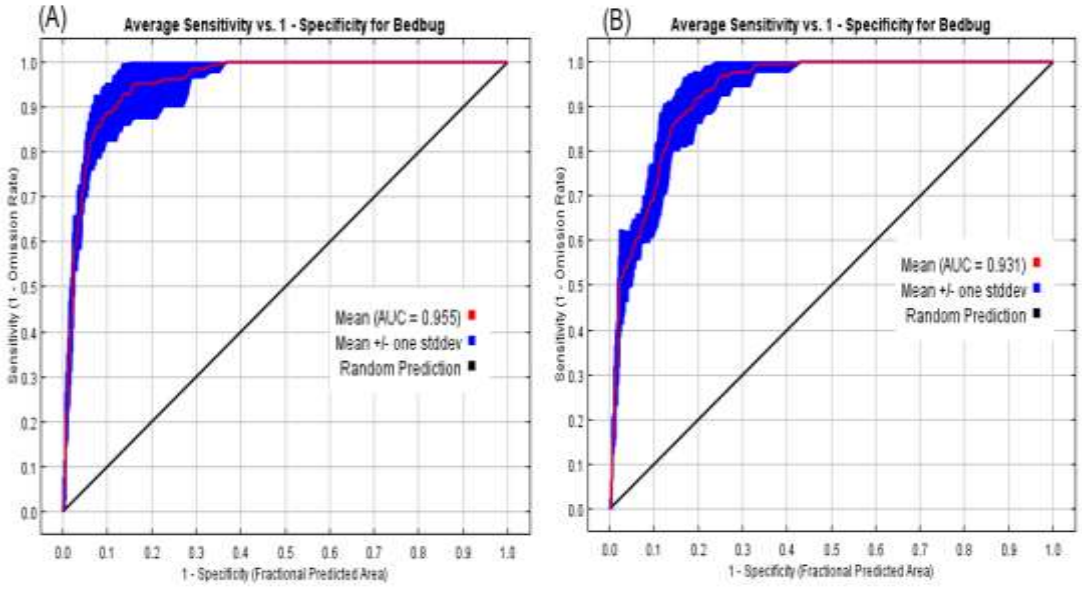


Figure 4.7: Receiver Operating Characteristic (ROC) curve verification of the predicted potential habitat for Bedbug in Kenya (A) and in Africa (B) by MaxEnt.

The jack-knife test demonstrated that land cover and Temperature Seasonality (bio_4) are the most important variable in determining the suitability of bedbug in Kenya (Fig. 4.8A); while in addition to the lates, Precipitation of Driest Month (Bio_14) and Precipitation of Warmest Quarter (Bio_18) variables significantly contribute to determining its suitability in Africa (Fig. 4.8B). In both cases, four bioclimatic variables: Bio_4, Bio_14, Bio_18 and Bio_7 (Temperature Annual Range (BIO5-BIO6)) have at least 0.5 value AUC, which indicated that they were the main bioclimatic factors affecting the potential distribution area of the bedbugs. The response of bedbugs to those four bioclimatic variables in Kenya is presented in Fig. 4.8C. In Africa, the distribution probability of the pest increased with the increase of the value of each bioclimatic variable within a certain range and decreased with the increase of the variables after a certain peak value (Fig. 4.8D).

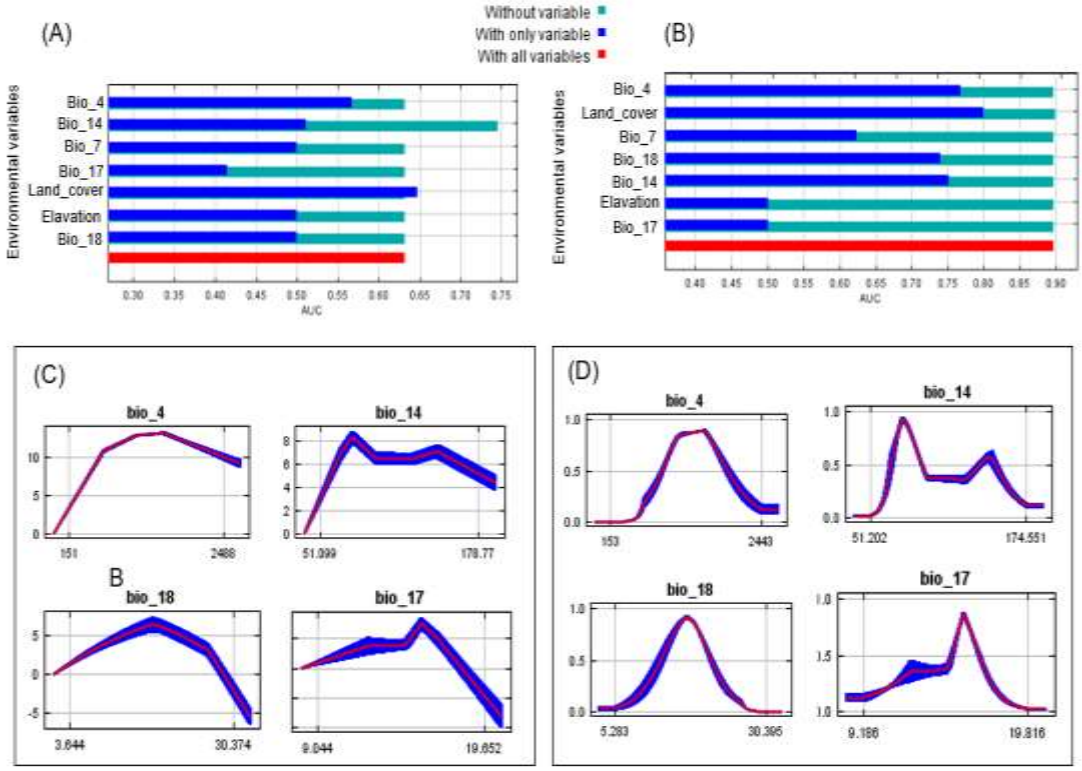


Figure 4.8: The relative importance of environmental variables for predicting the suitability habitat of bedbugs in Kenya (A) and Africa (B) and response curves between the probability and environmental variables in Kenya (C) and in Africa (D).

4.2 Diversity analysis

4.2.1 Phylogenetic analyses

The average size fragment of the mtDNA COI gene region amplified and sequenced from bedbug populations was 486 bp. A representative gel image is as shown (Fig. 4.9).

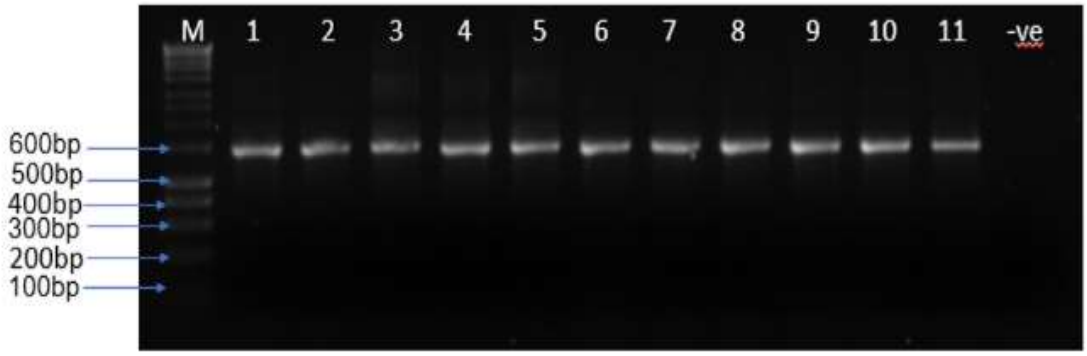


Figure 4.9: PCR results for 11 representative samples amplified using VF1d-t1 / VR1d-t1 primer pairs. (M: Hyper 100bp ladder; Lanes 1-5: Samples from Bomet; Lane 6-11: Samples from Machakos; Lane 12: Negative control)

All the 36 consensus sequences showed a percentage identity ranging from 99 to 100 after being blasted in the NCBI GenBank database. In these sampling populations, 66.7% bedbugs were identified as *Cimex hemipterus* while 33.3% as *Cimex lectularius* (Fig. 4.10).

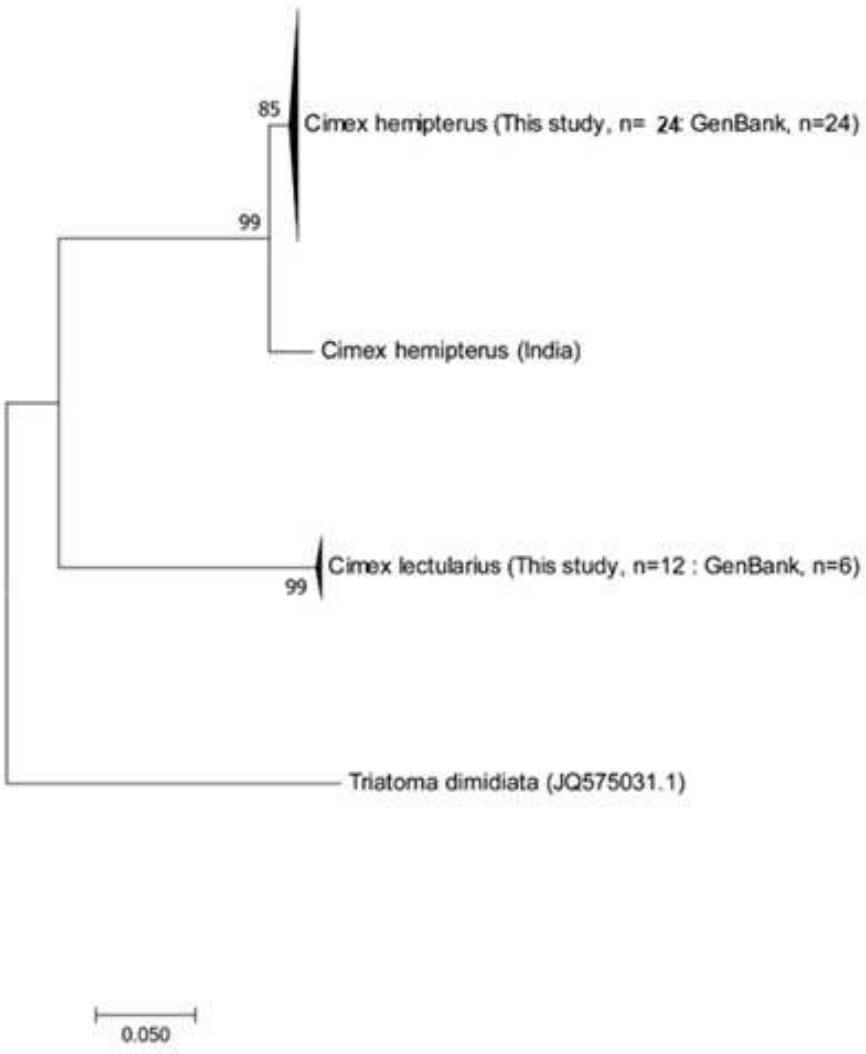


Figure 4.10: A condensed Maximum likelihood (ML) tree constructed in MEGA-X software inferred from sequences of the mitochondrial COI gene for 36 bedbug sequences from this study and 31 sequences from GenBank. Numbers at nodes indicate bootstrap values (%) obtained from 1,000 replications.

Based on the results of the phylogenetic tree (Fig. 4.10), the study populations were grouped into two major monophyletic clades. That is, *Cimex hemipterus* and *Cimex lectularius* bedbug strains. *Cimex hemipterus* strain harboured several haplotypes as

compared to *Cimex lectularius.* This was in concordance with the genetic diversity analysis of the two main clusters in this study (Table 4.1).

4.2.2 Polymorphism analyses

Having registered both the two species, DNA polymorphism analysis for the Kenyan bedbug population was performed using DnaSP as presented in (Table 4.1).

Table 4.1: Summary statistics for mtDNA genetic variation among Kenyan bedbug population.

Bedbug Strain	**n**	**h**	**Hd**	**π(k)**	**Fs**	**D***	**F***	**D**
C.lectularius	12	7	0.833 (±0.100)	0.00602 (±1.00118)	-1.434	-1.1062 NS	-1.17291 NS	-0.8204 NS
C.hemipterus	24	9	0.866 (±0.039)	0.00629 (±3.00579)	-1.162	0.03450 NS	0.07303 NS	0.12912 NS
Overall	36	16	0.924 (±0.022)	0.09765 (±47.4571)	11.850	1.71182**	2.44679**	2.70797**

n is the number of sequences, h is the number of haplotypes, Hd is haplotype diversity ± SD, π is nucleotide diversity, k is mean number of pairwise nucleotide differences, D* and F* are statistics as per Fu and Li (1993), Fs is Fu's statistic (Fu 1997), and D is Tajima (1989) statistic (** indicate statistical significance at 5%, NS=not significant).

There were 16 different haplotypes identified at an overall haplotype diversity of 0.924 (±0.022) out of which 7 belonged to *C. lectularius* strain while 9 to *C. hemipterus* strain. Further, Fu & Li's D* and F*, Tajima's D and Fu's F tests for *C. lectularius* were all negative though not significant. Overall, Fu & Li's D* and F* and Tajima's D tests were all significantly positive. Based on the two bedbug strains, the entire study identified 7 haplotypes (h) at a nucleotide diversity of 0.00602, and haplotype diversity of 0.833 for *C. lectularius* while *C. hemipterus* had 13 haplotypes at 0.00754 nucleotide diversity and 0.877 haplotype diversity (Table 4.1).

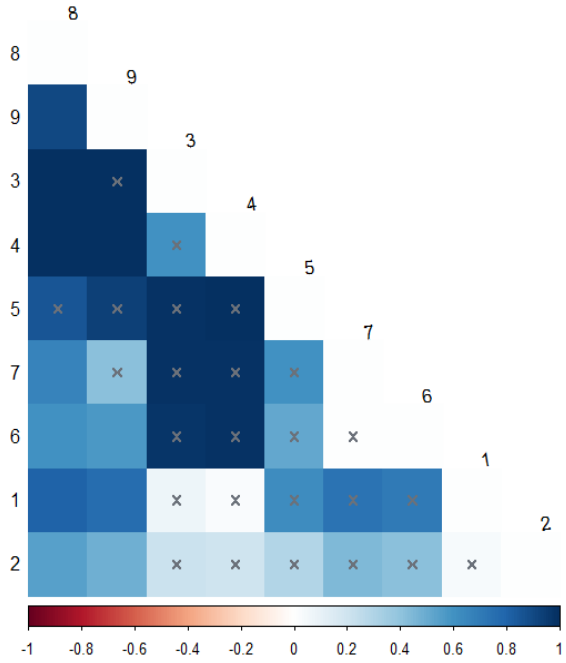
Moreover, population structure analysis based on the nine geographical regions (counties) of Kenya was as well performed (Table 4.2).

Table 4.2: Results of AMOVA between 9 Kenyan geographical groups (sampled counties).

Source of Variation	Sum of Squares	Variance components	Percentage Variation	P-Value
Among populations	1122.610	17.03444	66.59197	P<0.01
Within populations	538.390	8.54588	33.40803	
Total	1661.000	25.58032		

The genetic variation of the Kenyan population was mainly due to differences between individuals among populations (67%). Variability within populations accounted for (33%). The molecular diversity between individuals among the populations was significant with a p-value of $p<0.01$.

Additionally, AMOVA analysis results showed that Kisumu and Mombasa counties exhibited high variability. Kisumu county population significantly varied with Mombasa, Bomet, Kericho, Nairobi, Makueni, Narok and Machakos counties. Also, Mombasa County population significantly varied with Kericho, Makueni, Narok and Machakos counties (Fig. 4.11).



Legend:

1. Narok	5. Kiambu
2. Machakos	6. Makueni
3. Bomet	7. Nairobi
4. Kericho	8. Kisumu
5. Kiambu	9. Mombasa

Figure 4.11: A correlation plot representing the genetic variability among Kenyan sampling populations (labelled 1 to 9). Crosses (x) indicate insignificant differences between populations, suggesting homogeneity.

4.3 Insecticide resistance

The presence and distribution of V419L VGSC resistance gene in 277 bed bug samples collected from dwellings across Kenya (nine counties), were assessed. Out of the 277 samples, 104 (37.5%) samples were successfully amplified by the BBparaF1/ BBparaR1 primer and 79 (28.5%) samples showed the presence of the gene (Fig. 4.12,Table 4.3). Out of 25 samples screened from Narok County in Kenya, none of them indicated presence of the gene.

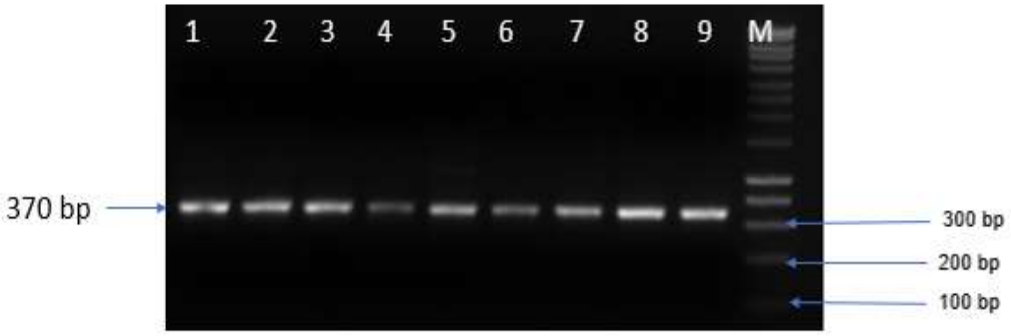


Figure 4.12: PCR results for 9 (nine) representative samples amplified using allele-independent primer pairs, BBparaF1/ BBparaR1. (M: Hyper 100bp ladder; Lanes 1-5: Samples from Kericho; Lane 6-9: Samples from Makueni)

Table 4.3: A summary of successful PCRs and sequences

	N	**(%)**
Evaluated samples	277	100
PCRs	104	37.5
Sequences	79	28.5

4.4 Gut microbiome

4.4.1 Library size and cumulative abundance

The cumulative abundance of the bacterial genomes in these bedbug sampling populations were presented using a stacked bar graph. The overall results showed that *Wolbachia* (68.42%) was the most abundant genera harboring bedbugs, followed by *Klebsiella* (4.90%) and *Escherichia* (3.31%) (Fig 4.13). Across the sampled counties in Kenya, *Wolbachia* (71.09%), *Escherichia* (3.50%) and *Klebsiella* (3.16%) were recorded as the dominant gut microbes (Fig 4.13B). The relative cumulative abundance of bacterial genera at the species level was presented in (Fig. 4.13A). For *Cimex lectularius*, the predominant genera were *Wolbachia* (62.67%), *Escherichia* (7.48%), and *Sodalis* (6.25%), while for *Cimex hemipterus*, *Wolbachia* (70.72%), *Klebsiella* (5.50%) and *Edwardsiella* (2.77%) were the most abundant (Fig 4.13A).

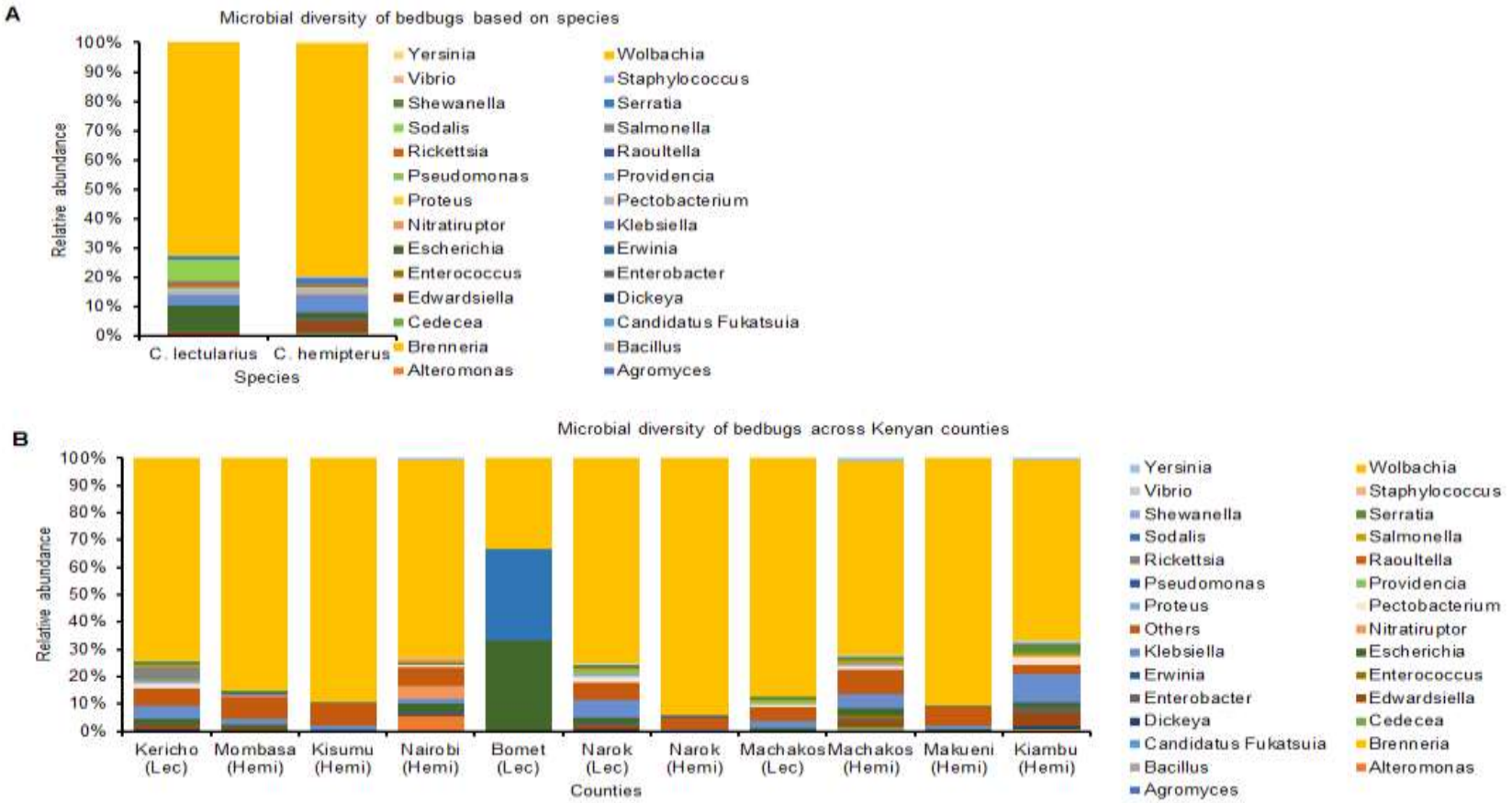


Figure 4.13: Taxonomic composition and percentage of reads of bacteria at the genus level of the bacteria community in bedbug's gut microbiome from the two identified common bedbug species (A) and nine (9) counties of Kenya (B). (Lec, C. lectularius; Hemi, C. hemipterus).

4.4.2 Bacterial community profiling

4.4.2.1 Alpha diversity

The analysis showed that Bomet had the highest evenness of about 1.0 while Narok (0.09) had the least. The average species evenness of the sampled counties ranged between 0.1-0.4 (Fig 4.14B). Kericho county registered the highest bacterial species richness of 227, while the least was recorded in Bomet with 4. The average species richness ranged between 100-200 (Fig 4.14A). Machakos county recorded the highest bacterial Shannon index of 1.78, while the least was observed in Narok with 0.44 (Fig 4.15, Appendix VI).

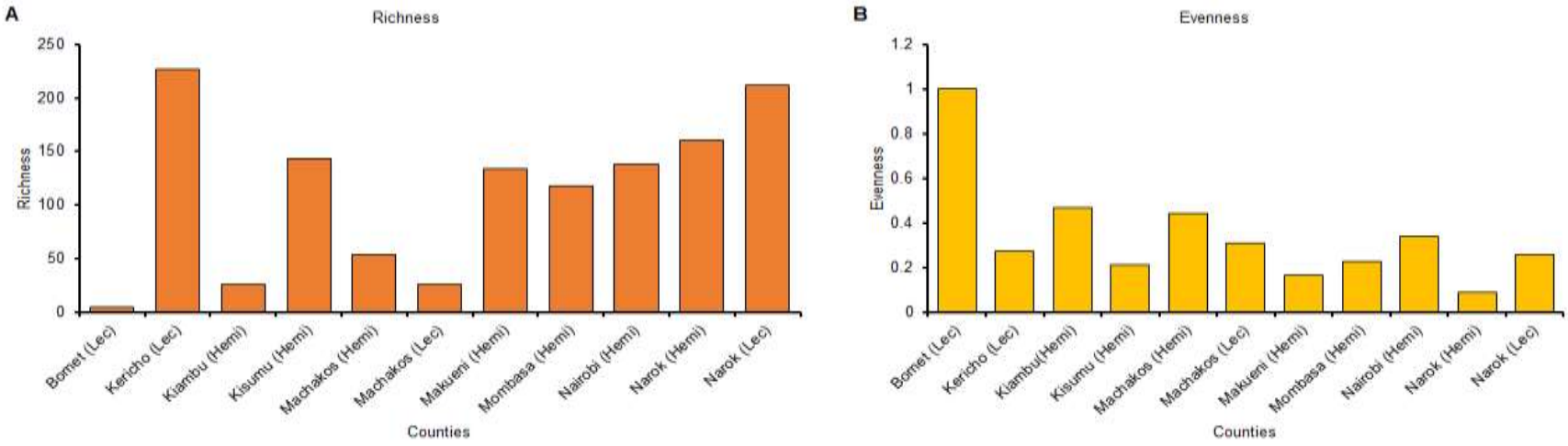


Figure 4.14: Alpha-diversity statistics showing evenness and species richness across bedbugs collected in nine (9) counties of Kenya. The samples are represented on the X-axis, and their estimated diversity is represented on the Y-axis. (Lec - *C. lectularius*; Hemi - *C. hemipterus*).

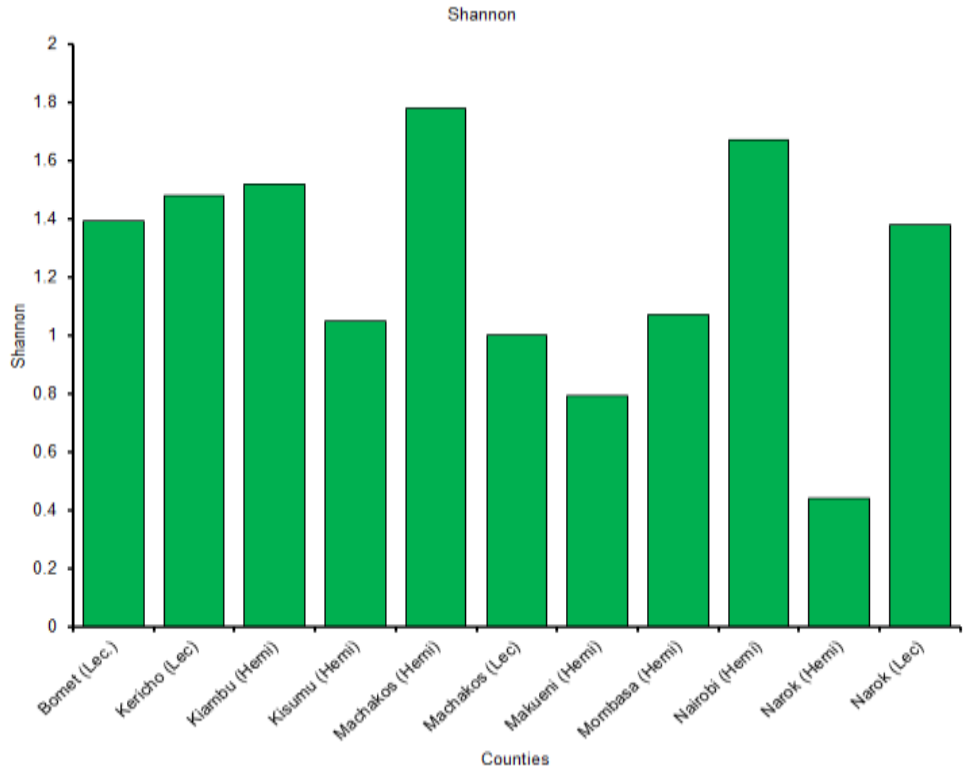


Figure 4.15: Alpha-diversity statistics showing Shannon-Wiener index across bedbugs collected in nine (9) counties of Kenya. The samples are represented on the X-axis, and their estimated diversity is represented on the Y-axis. (Lec - *C. lectularius*; Hemi - *C. hemipterus*)

4.4.2.2 Beta diversity

Bray Curtis dissimilarity index was used to estimate beta diversity. The results showed several distinct clusters from the sampled counties in Kenya (Fig. 4.16). Counties infested with *C. hemipterus* showed that the highest interpopulation diversity was between Kiambu and Narok populations (96.44%) while the least was recorded between Makueni and Kisumu (14.09%) (Fig. 14.16A). Moreover, *Cimex lectularius* infested counties of Kenya revealed that the highest interpopulation diversity was between Kericho and Bomet populations (99.89%) while the least was recorded between Kericho and Narok (11.20%) (14.16B, Appendix VII).

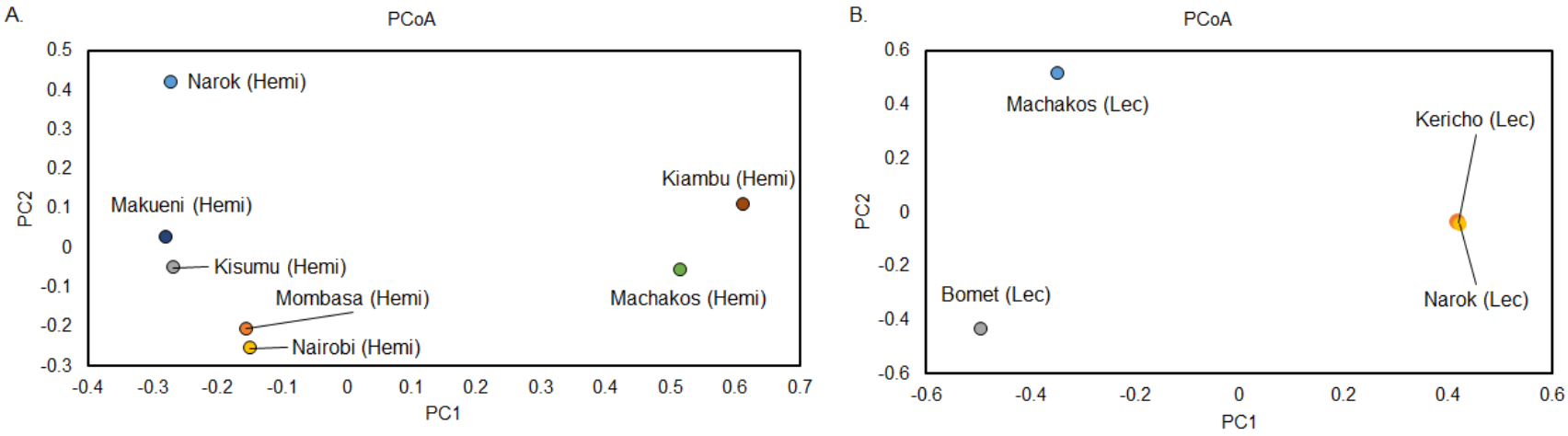


Figure 4.16: Principal Coordinate Analysis (PCoA) plot estimated using Bray Curtis dissimilarity index of bacteria communities of bedbugs collected from nine (9) counties of Kenya. (Lec - *C. lectularius*; Hemi - *C. hemipterus*).

4.4.2.3 Abundance of pathogenic bacteria

A total of 20 pathogenic genera were recorded in this study. Majority of them were in very low abundance, with the predominant ones being *Escherichia* (3.31%), *Salmonella* (0.72%), *Yersinia* (0.45%), *Vibrio* (0.44%), *Pseudomonas* (0.38%) and *Rickettsia* (.036%) (Appendix VIII).

CHAPTER FIVE

DISCUSSION

5.1 Management approaches and socio-economic impacts of bedbug infestations in households

Community-wide perception and knowledge assessment on bedbugs revealed an ample awareness of the population and several interesting bedbug infestation patterns. The respondents confirmed to experience a resurgence of bedbug infestations and had high infestation rate. Bedbug resurgence and infestations have recently been reported globally as a significant health problem (Deku *et al.*, 2021). In 2018, Kenya reported 4,000 bedbug-infested homes, therefore the rise of infestations parallels that with other African nations and the entire world (Fourie *et al*., 2018). Moreover, the pest has been reported as a potential public health hazard and a socioeconomic burden with approximately USD 2,500 - 3,000 USD per infestation spent in disinfection and replacement of infested belongings (Davies *et al*., 2012b). The 49.8% of females versus 50.2% of male respondents recorded during the survey of this study was an important indicator to avoid biasness of gender in the variables of our data.

The high prevalence of bedbugs is directly related to psycho-socio-economic-health and hygienic effects. Overall, 80% of the respondents during our survey perceived bedbugs as a weird occurrence as compared to 20% who perceived them as a normal pest in society. This is because the pest is associated with poor economic status and an unhygienic environment (Kaliyaperumal Karunamoorthi *et al*., 2015). However, bedbugs affect all socioeconomic backgrounds with no poverty level correlation (Wu *et al*., 2014a). This phenomenon can be attributed to various factors including infestation in public transport such as airplanes, ships (How & Lee, 2010) and vans (Potter, 2006). Besides, human habitats, especially the permanent ones like residential premises, serve as potential reservoirs for the ectoparasites. In contrast, public accommodation acts as

converging and diverging points for new infestations. Nearly 69% of mild bite symptoms were reported as predominant across the study areas. This could have been due to a lack of inflicted pain and immediate signs since a delay of the response of up to nine days has been documented in the past (Hans, 2015). On the other hand, 29% severe bite reactions due to high sensitivity are reported to have been treated by visiting a medical facility or acquiring antiallergics or painkillers over the counter. The results of our study concur with other studies, that have documented the use of oral antihistamines, topical corticosteroids, and topical emollients to treat bedbug bites (Hwang *et al*., 2005). Furthermore, not always all the bites result from bedbugs, so every bite should be confirmed. Bedroom/mattresses, furniture, cracks/crevices, and clothes were the most infested areas in the infested houses. This kind of distribution is probably due to frequent dispersing since the ectoparasite establishes new harborages once the habitat does not favor them anymore (Pfiester *et al*., 2009). Gangloff-Kaufmann et al. (2006) and Hwang *et al*., (2005) have reported bedbugs to mainly inhibit mattresses and beddings (Gangloff-kaufmann *et al*., 2006; Hwang *et al*., 2005). In a resource-limited setting, it is quite obvious that the economically and socially disadvantaged groups share a significant burden of infestation due to limited or no financial capabilities to disinfest. This was observed in this study where some respondents disinfected their habitat/materials once per year and some could not even afford the cost of the treatment.

In order to understand the dynamics of bedbug infestation in residential community, this study used a simple metapopulation approach and proposed SIT model by having two cases: a basic model for homogeneous houses and model for heterogenous houses. The infestation dynamic and interactions of bedbug species in a residential community model showed a rapid mobility of bedbug from one house to another where about 94% of houses in the community were infested after ten months post introduction of the pest in the community. Bedbugs do not fly since they do not have wings. They are only able to crawl and move short distances within an infected area, and slowly spread to other rooms in the house. Therefore, the high or rapid spread of the pest in the community is

mostly attributed to human factors. Bedbugs then rely upon humans to help transporting them to new areas and locations where they are free to set up and establish new infestation. People get bedbugs from being at places where bedbugs are, as they are hitch-hikers and largely dependent upon human mobility to travel from one place to another. Bedbugs can climb into luggage or other belongings and are then brought home. They can also move around by finding their way into purse, backpacks, clothing, suitcases, briefcases, and jackets and one can carry them to other locations by simple visit of infested neighbouring house. When considered the scenarios of heterogenous of bad and good houses, the number of infested good house (Ig) was much less as compared to the infested bad houses (Ib). This could be explained by the fact that low income, less education, unemployment, living in group homes and staying at homeless shelters are perceived as some of the factors attributed to bedbug infestations (Sheele, 2021). This confirmed the hypothesis that new or improved houses labelled as "good" houses do not provide very suitable conditions for the survival of bedbugs.

Further, the spatial distribution analysis of bedbugs in Kenya and in Africa results showed that the habitat suitability of *Cimex spp.* (Hemiptera: *Cimicidae)* predicted for the current scenario agreed strongly with the ground observations. According to Koppen-Geiger climate classification (Beck *et al*., 2018), the tropical climate has been grouped into three including: tropical rainforest, tropical monsoon and tropical savannah. In Kenya, this climate is mainly found along the coastal region and lakeside (Lake Victoria), although fragmented in some other parts of the country. Our distribution prediction results are in tandem with this classification since *Cimex spp*. habitat suitability has been predicted as optimal and highly suitable in these regions. Temperate climate, which covers a relatively larger part of Kenya (central, western, and southern), has been predicted as areas of optimal and high habitat suitability for the pest. Also, the northern and northeastern parts of Kenya, usually known to be semi-arid, have been predicted as a suitable habitat for Cimex spp. in our study. In general, this study demonstrated Kenya as entirely a potential suitable habitat for *Cimex spp*. since none of

its regions has been predicted as marginal or unsuitable habitat. Furthermore, the prediction across Africa concurred with Koppen-Geiger climate classification (Beck *et al*., 2018). The northern arid and semi-arid part of Africa has been predicted as a suitable habitat for the pest. Tropical climates, found in central and western Africa regions, have been predicted to have optimal habitat suitability. However, some regions in this area have been predicted as highly suitable for bedbugs. Finally, southern Africa, which has a subtropical and temperate climate, has been predicted to be a region of suitable and highly suitable habitats. Therefore, this study delineates Africa as a potential habitat of Cimex spp. *(Hemiptera: Cimicidae)* as well.

The habitat predicted by our model is similar to that of the known occurrence point; however, there was some observed deviations from the actual occurrence areas of the pest. Besides the bioclimatic variables as initial predictors, other non-climatic factors such as human activities, control measures, host and natural enemy affect species habitat suitability (R. Wang *et al*., 2020). Generally, models are just an estimate of a species potential distribution and cannot replace fieldwork but rather can be an indispensable tool for data exploration meant to help identifying knowledge gaps and provide guidance for fieldwork design (Elith *et al*., 2006).

As the pest became a serious public health nuisance with its high population dynamics and high potential geographical distribution, bedbug merits serious management approaches. Residents have reported to have applied various control and management practices. This comprised use of chemical method, cultural method, and botanicals but respondents highlighted chemical method as the most effective. Furthermore, the model simulations' outputs indicated that use of chemical control method was more effective than using other control strategies. This can be attributed to the fact that insecticides are reported as a powerful weapon in eradicating domestic pests and fighting against other vector diseases, as well as the existence of a broad range of insecticides, including pyrethroids, neonicotinoids, pyrroles, organophosphates, and carbamates (Dang *et al*., 2017; Kaliyaperuma Karunamoorthi & Yirgalem, 2014; Kaliyaperumal Karunamoorthi

& Tsehaye, 2012). In our study, some of the predominant insecticides used across the surveyed counties contain Chlorpyrifos, Carbaryl, Fipronil, Pyrethrin, Imidacloprid, and Dichlorvos as active compounds. According to the World Health Organization (WHO), Chlorpyrifos 480g/l, Carbaryl 7.5%, Fipronil, 6% Pyrethrin and Imidacloprid 200g/l, all have been classified as class II (moderately hazardous) while Dichlorvos 1000g/L as class 1b (highly hazardous) (WHO, 2020). Therefore, the haphazard and unsafe use of those pesticides could result in serious negative effects on the environment, human and animal health. In addition, most of the residents are reported to use higher concentrations of these pesticides than stipulated and not adhering to the label rates. This puts them at an increased risk of indirect health problems such as toxicological health issues (Balster, 2011; Deku *et al*., 2021). Furthermore, insecticide overuse has resulted in ever increasing organophosphate concentrations in water bodies (Dabrowski et al., 2002), which puts the residents at a risk of chronic diseases such as asthma, kidney disorders, hormonal disorders and other insecticide-related toxicological disorders (Deku *et al*., 2021). Due to the toxic nature of insecticides and unregulated application practices, appropriate awareness creation is very important to be conducted.

Fortunately, the outputs of model simulations showed that the combination of chemical methods with other control methods was considerably much more effective in reducing the number of infested houses in the community compared to even the use of chemical method only and concentrating on the treatment of only bad houses was the better option. Therefore, integrated pest management (IPM) strategy by combining different control methods should be a better intervention approach in control of bedbugs as it also limits dependence on pesticides and reduces risks for humans and the environment. Cooper *et al*. (2015) have reported that adopting a complex-wide bedbug IPM program, proactive monitoring, and biweekly treatments of infested houses utilizing non-chemical and chemical methods can successfully reduce infestation rates to very low levels.

5.2 Genetic diversity of bedbugs

Phylogenetic analysis and nucleotide variation are common molecular techniques that have been implemented successfully in the past for diversity analysis and resolving phylogeographic studies (Seri Masran & Ab Majid, 2017). This study focused on bedbugs' population genetics and infestation dynamics by utilizing mtDNA, a reliable tool of choice (Szalanski *et al*., 2008).

It was observed that there were 16 haplotypes among the 36 samples of the bedbug examined. On further dissection of our data, the *C. lectularius* strain had 7 haplotypes while *C. hemipterus* strain had 9. In this study, both species were detected as revealed by phylogenetic tree analysis and supported by a high mean pairwise distance of 0.261. The two clades were comprised of homogeneous populations, which is in concordance with a low value of the mean pairwise distance of 0.01 between the individual samples of each clade. The clustering agrees with other previous studies as observed by (Tawatsin *et al*., 2013). The fewer number of haplotypes (7) of *C. lectularius* strain as compared to the *C. hemipterus* strain (9) can be attributed to limited genetic diversity and elevated levels of inbreeding (Seri Masran & Ab Majid, 2017). This is in agreement with Booth *et al*. and Saenz, 2012 who documented inbreeding as a major occurrence in the *C. lectularius* strain of bedbugs (Booth *et al*., 2012; Saenz *et al*., 2012). Additionally, *C. lectularius* has been recorded to possess mechanisms of purging deleterious mutations that causes inbreeding depression unlike with *C. hemipterus* (Mohammad *et al*., 2020).

Negative values of Fu's *Fs* are attributed to an excess of rare haplotypes, suggesting a possibility of population expansion in the recent past (Fu, 1997). Both *C. lectularius* and *C. hemipterus* recorded negative Fu's *Fs* values, therefore, revealing a probability of population expansion for the two strains. Overall, Tajima's D test statistic (1.67227) in our study indicates a lack of an excess of recent or rare haplotypes. This suggests that population contraction had occurred instead of expansion of haplotypes (Tajima, 1989). Interestingly, negative values of Tajima's D test statistic support a recent population

growth for *C. lectularius* strain, unlike with *C. hemipterus*. Moreover, *C. lectularius* evolutionary tests of Fu & Li's D* and F* (-1.10623 and -1.17291, respectively) indicates an excess of rare haplotypes which can be attributed to positive selection sweeps, recent population growth or background selection (Fu & Li, 1993).

The results were fascinating having recorded both of the two bedbug strains. The findings were consistent with other findings that have recorded both species coexisting in the same area (Benkacimi *et al*., 2020). Also, *C. hemipterus* (tropical bedbug) has been reported to exist in sympatry with *C. lectularius* in Australia and Florida (Dang *et al*., 2017a) and as well in non-tropical climates such as in France and Russia (Benkacimi *et al*., 2020). Mombasa and Nairobi populations were all identified as *C. hemipterus* which clustered with GenBank samples from Malaysia and Singapore. This common mtDNA haplotype observed may be attributed to international travels. In 2014, international travels were recorded at 1.1 billion, with an estimated increase to 1.8 billion by 2030 (Delaunay & Pharm D, 2012). Phylogenetic tree analysis showed that the population of *C. lectularius* grouped with the GenBank Europe population, where the strain has been reported to have widely established itself (Benkacimi *et al*., 2020) thus, implicating it to international connections and travels. Therefore Kenya, a hub of tourism, might emerge as a hotspot for expanding bedbug infestations, particularly *C. lectularius*.

The study populations from Narok exhibited high homogeneity with Bomet and Kericho populations, a scenario attributable to the high frequency of travel to and from the three neighbouring counties. However, there was lack of correlation between genetic distance and physical distances among the sampling populations since our findings indicate some instances of high homogeneity among geographically disjunct regions. This observation was in tandem with our AMOVA analysis where interconnected populations were as significantly differentiated as those further away, suggesting saturation of genetic differentiation.

In this study, the relatively moderate mtDNA diversity among the two identified bedbug species can be accounted for by the limited sample size and geographical scale. Therefore, future population genetics studies will require to cover micro and macro geographical scales within a well-defined hierarchical sampling design.

5.3 Bedbug insecticide resistance

In order to understand the dynamics of bedbugs' insecticide resistance, this study provides evidence for relatively high frequency of V419L target region of VGSC resistance gene. Sequencing consistently confirmed the results observed through PCR by recording 79 (28.5%) bedbug samples showing presence of the resistance gene. This suggests that the rate of pyrethroid resistance is relatively high and other natural populations could be developing resistance to pyrethroids and other insecticides that are being applied. The presence of the V419L target region of VGSC resistance gene in our study supports the findings of previous studies that have documented V419L, L925I, L899V, M918I, D953G and L1014F as the common kdr mutations in bedbugs (Punchihewa *et al*., 2019). Further, the distribution and frequency of the V419L mutations has revealed an alarming pattern of geographical distribution across United States, Australia, Japan, Europe, and Israel (Balvín & Booth, 2018). For example, in United States insecticide resistance from V419L mutations was recorded at ~41% (Zhu *et al*., 2010).

5.4 Pathogenic loads in the bedbug gut microbiome

Gut microbiome analysis of bedbugs in this study showed that *Wolbachia* comprised the largest fraction of the microbiome (~68.42%) and was present in all sampled individuals and locations. *Wolbachia* infections and other endosymbionts such as *Acinetobacter, Citrobacter, Pseudomonas,* and *Yersinia* are common in Cimicidae (Sakamoto *et al*., 2006; Soh & Veera Singham, 2022). Therefore, the high frequency of *Wolbachia* found in this study is not surprising but rather provides evidence that nanopore long-read

sequencing of the full-length 16S metagenome is congruent with expectations. As a predominant obligate endosymbiont, *Wolbachia* is vertically transmitted and could be found in several cell types (Lim & Ab Majid, 2021). However, the exact role of *Wolbachia* in bedbugs is yet to be fully described having previously been associated with host reproduction, provisioning of B vitamins (biotin and riboflavin (Bellinvia *et al*., 2020; Hosokawa *et al*., 2010; Nikoh *et al*., 2014), mediating insecticide susceptibility and increasing tolerance to insecticides in insects (Li *et al*., 2018).

Beta diversity estimated based on Bray-curtis showed significant differences between the sampled counties. This result is attributable to the different states of the bedbug samples at the time of sampling. That is, some might have fed while others might have not. Starved bedbugs have been reported to have a more diverse and abundant microbial community compared to the fed ones due to host-driven mechanisms such as PH changes, production of antimicrobial proteins and reduced mucus secretion on the host gut lining (Lim & Ab Majid, 2021). Tchioffo *et al* (2016) revealed that mosquitoes experienced lower bacterial diversity and abundance compared to the starved ones probably due to the digestive process that killed the symbiont bacteria (Tchioffo *et al*., 2016). Nevertheless, there exist other contrasting studies.

In this study, several bacterial taxa were recorded across all sampling populations. Further, some were shared between the two bedbug species (*C. lectularius* and *C. hemipterus*). These results suggest probable vertical transmission of the endosymbionts and their critical role in providing supplementation and maintenance of their host homeostasis and immune system (Engel & Moran, 2013; Weiss *et al*., 2011). For example, *Pseudomonas*, a generalist endosymbiont, could be found in several insects such as mosquitoes and is always linked to detoxifying activities in insects' guts (Lim & Ab Majid, 2021).

Among the objectives of our study, one was to detect human pathogenic bacteria in bedbugs. Although with no casual evidence, past studies have reportedly identified 45

pathogens (bacteria, protozoans, viruses, fungus) that have the potential to be transmitted by bedbugs (Fisher *et al*., 2019). Furthermore, bedbug bites and excrements poses a number of health risks including asthma, anaphylaxis, secondary infections and adverse impact on quality of life (Kakumanu *et al*., 2020; Meriweather *et al*., 2013). This study uncovered at least 20 genera to which known or putative human pathogens belong, including *Escherichia*, *Salmonella*, *Pseudomonas, Yersinia*, *Vibrio*, *Rickettsia, Bacillus*, *Streptococcus* and *Staphylococcus* among others. Therefore, species-specific screening is warranted for the identification of bacterial species and strains of interest to human health.

CHAPTER SIX

CONCLUSION AND RECOMMEDATIONS

6.1 Conclusion

This study successfully identified the two common bedbug species; *C. lectularius* and *C. hemipterus*. This calls for rapid action, immense awareness campaigns accompanied by technological innovation and national, regional, and international cooperation to develop a long-lasting, suitable and sustainable solution. This study successfully modelled the mobility and infestation dynamics patterns for *Cimex spp*. It also provides the first predicted potential habitat distribution map for the bedbug species in Kenya and Africa. The predicted potential habitat distribution map of *Cimex spp*. could therefore help in planning, monitoring, and identifying top-priority survey sites; and integrated pest management approach is highly recommended in the management of the pest as the model showed that the combination of chemical methods with other control methods was considerably much more effective in reducing the number of infested houses and the pest pressure in the community compared to the use of chemical methods only. Furthermore, the study reports the presence of insecticide (pyrethroids) resistance and bedbug gut microbiome composition. These findings expand the knowledge about diversity, current and future potential habitats, community perceptions and knowledge, insecticide resistance levels and gut microbiome contribution in relation to human pathogenic bacteria.

6.2 Recommendations

Based on the findings from this study, the following key recommendations are emphasized:

1. Extensive campaign on the use of integrated pest management approach in the management of the pest.

2. Further population genetics studies that will cover micro and macro geographical scales within a well-defined hierarchical sampling design.
3. The combination of chemical methods with other control methods in the disinfestation of bedbugs.
4. Further species-specific screening to identify bacterial species and strains of interest to human health.

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APPENDICIES

Appendix I: questionnaire modules for the assessment of bedbugs outbreaks, distribution, management and control practices among residents in different counties in Kenya

Introductory statement:

"Dear Sir/madam, I work for the International Centre of Insect Physiology and Ecology (*icipe*). We are conducting a survey to study the level of infestations, economic impact, perceptions, knowledge, public awareness and practices regarding bedbugs outbreaks, distribution, control and management around the country. Your response to these questions would remain anonymous, and taking part in this study is voluntary. We request that you answer the questions as accurately and honestly as possible so that our understanding and future activities/actions are then based on addressing the real pest control, management and problems faced by residents like yourself. If you choose not to take part, you have the right not to participate and there will be no sequences. Thank you for your kind co-operation."

MODULE 1. HOUSEHOLD AND VILLAGE IDENTIFICATION

1.1 Household Identification	**Code**		**1.2 Interview details**	**Code**
1. County			**14**. Date of interview (dd/mm/yyyy):	/ / 2020
2. Sub-county			**15.** Time started (24 HR)	
3. Ward			**16**. Name of enumerator	
4. Location			**17**. Name of supervisor:	
5. Village:			**18.** Name of data entry clerk	
6. Age of respondent				
7. Name of household head (three names):				
8. Sex of household head	1=Male			
9. Name of the respondent (three names):			**GPS reading of homestead**	
10. Sex of respondent	1=Male		**19.** Way point number	

11. Name of respondent's spouse		**20.** Latitude (North)	
12. Cell phone number of household head		**21.** Longitude (East)	
13. Cell phone number of the spouse:		**22.** Altitude (meter above sea level)	

MODULE 2: HOUSEHOLD COMPOSITION, CHARACTERISTICS AND HOUSING CONDITIONS

2.1 HOUSEHOLD COMPOSITION AND CHARACTERISTICS (Household members: persons who live together and eat together from the same pot (share food), including hired labour, students and spouse living and working in another location but excluding visitors)

ID CODE	Name of household member [Start with respondent]	Sex 1=M 0=F	Relationship to the household head **CODE 1**	Age (complete years; 0 if less than 1 year)	Marital status? **CODE 2**	Education (years) **CODE 3**	Primary occupation **CODE 4**	How many months in the past year was [NAME] present in the household?	Number of children living in the same household (under age of 18yrs)	Number of children living in the same household (above age of 18yrs)
	AA1	**AA2**	**AA3**	**AA4**	**AA5**	**AA6**	**AA7**	**AA8**		
1										

2										
3										
4										

CODE 1		CODE 2	CODE 3	CODE 4	
1.Household head	6. Grandson/granddaughter	1.Married living with spouse	0. None/Illiterate	1.Farming (crop+ livestock)	5.Casual labourer off-farm
2.Spouse	7.Other relative	2.Married living without spouse	1. Adult education or 1 year of education	2.Salaried employment	6.School/college child
3.Son/daughter	8.Hired worker	3.Divorced/separated	* Give other education in years (e.g. 2 yrs for std 2, 8 yrs for class 8 etc)	3.Self-employed off-farm	7.Non-school child
4.Parent	9. Other, specify……	4.Widow/widower	100. Religious education	4.Casual labourer on-farm	8. Other, specify……..
5.Son/daughter-in-law		5.Never married			

MODEL 3: BED BUG CONTROL AND MANAGEMENT PRACTICES

3.1. Have you experience bed bugs menace/nuisance before?

1. Yes ( )
2. No ( )

3.2. If yes, for how long have you encountered bed bugs?

1. 1-5 years ( )
2. 6-10 years ( )
3. 10-15 years ( )
4. 16-20 years ( )
5. Above 20years ( )

3.3. What are some of the methods you have tried to manage them?

1. Burn with hot water ( )
2. Exposing infested items to sunlight outside ( )
3. Use of pesticides ( )
4. Any other specify ( )

3.4. Which of the above-mentioned methods has been the most effective?

3.5. On the above-mentioned effective method(s), was it a one-time treatment or on repeated occasions?

1. Once ( )
2. Repeated ( )

3.6. How many pesticides have you tried so far to eradicate the bugs?

3.7. On the above-mentioned types, which one would you recommend as the most effective pesticide?

1. None ( )
2. Any other specify ( )

3.8. After fumigation, how long does it take for the bugs to disappear?

1. 1 month ( )
2. 2 months ( )
3. 3 Months ( )
4. Above 4 months ( )

3.9. Have you ever relocated before because of the nuisance pest?

1. Yes ( )
2. No ( )

3.10. If YES, did it work for you?

1. Yes ( )
2. No ( )

3.11. How adverse are the effects of the bed bug bites?

1. None ( )
2. Mild ( )
3. Severe ( )

3.12. If severe, how did you manage (medication) them?

3.13. Which pesticide is commonly available and pocket friendly do you often use?

3.14. Was the pesticide effective enough?

1. Yes ( )
2. No ( )

3.15. If NOT, which measures did you take?

3.16. In the household items, where is the highest incidence of the bugs?

1. Furniture ( )
2. Mattresses/bedroom ( )
3. Cracks/Crevices ( )
4. Clothes ( )
5. Others Specify

3.17. Does the infestation affect your self-esteem and social life in general?

1. Yes ( )
2. No ( )

3.18. How do the general community around you perceive bed bug infestations?

1. Weird ( )
2. Normal ( )
3. Any other specify ( )

3.19. After what duration do you notice the bug bites?

1. Immediately ( )
2. 30mins-1hr ( )
3. Any other specify ( )

3.20. At what time of the day are the bugs most active?

1. Morning ( )
2. Midday ( )
3. Night ( )

MODULE 4

4.1 INFRASTRUCTURE (all distances in walking minutes)

4.1.1 Give the estimated distance to the following community infrastructure and services centers from your residences

Infrastructure	**Distance (Minutes)**
Village market	
Nearest source of insecticides and pesticides (dealers)	
Nearest neighboring household	
Nearest health center	

Time finished interview (24 HR) …….

Thank you very much for your time and participation (Please remember to thank the farmer genuinely)

The enumerator to answer section 14 below privately immediately after the interview

12.1 Did you ask questions properly? [____] 0=No 1=Yes

12.2 Overall, how did the respondent give answers to the questions [___]

1=willingly	2=reluctantly	3=with persuasion	4=it was hard to get answers

12.3How often do you think the respondent was telling the truth [____]?

1=rarely	2=sometimes	3=most of the times	4=all the time

Checked by Supervisor:

I (supervisor)_____certify that I have checked the questionnaire to be sure that all the questions have been answered, and that the answers are legible.

Signed: _____

Appendix II: Study of respondents with bite effects, community perception, self-esteem impact, disinfestation frequency, infested areas, duration of encounter, post-disinfestation disappearance duration, control strategy and cases of relocation due to infestations.

Bedbug knowledge, infestation and control practices variables		n	(%)
Bite effect			
	None	21	2.3
	Mild	620	68.9
	Severe	259	28.8
Community perception			
	Weird	719	79.9
	Normal	180	20
Disinfestation frequency			
	Once	92	10.2
	> once	808	89.8
Most infested areas			
	Bedroom/Mattresses	306	34
	Furniture	252	28
	Cracks/crevices	216	24
	Clothes	126	14
Duration of encounter			
	1 - 5 Years	689	76.6
	6 - 10 Years	174	19.3
	11 - 15 Years	22	2.4
	16 - 20 Years	14	1.6
	> 20 Years	1	0.1
Post-disinfestation disappearance duration			
	1 Month	464	51.6
	2 Months	218	24.2
	3 Months	92	10.2
	≥ 4 Months	125	13.9
No. of insecticides used			
	None	77	8.6
	One	333	37
	Two	367	40.8
	Three	102	11.3
	Four	19	2.1
	> Four	1	0.1
Control strategy			
	Chemical and cultural[1]	714	79.3
	Cultural only[2]	93	10.3
	Chemical only[3]	83	9.2
	Botanicals[4]	10	1.1
Have you ever relocated because of an infestation?			
	Yes	456	50.7
	No	444	49.3
Does the infestation affect your self-esteem and social life in general?			
	Yes	871	96.8
	No	29	3.2

Appendix III: List of environmental variables

Variables	Abbreviation	Unites
Isothermality (BIO2/BIO7) (* 100)	bio3	°C
Temperature Seasonality (standard deviation *100)	bio4	°C
Max Temperature of Warmest Month	bio5	-
Min Temperature of Coldest Month	bio6	°C
Temperature Annual Range (BIO5-BIO6)	bio7	°C
Mean Temperature of Wettest Quarter	bio8	°C
Mean Temperature of Driest Quarter	bio9	°C
Mean Temperature of Warmest Quarter	bio10	°C
Mean Temperature of Coldest Quarter	bio11	°C
Precipitation of Wettest Month	bio13	Mm
Precipitation of Driest Month	bio14	Mm
Precipitation of Wettest Quarter	bio16	Mm
Precipitation of Driest Quarter	bio17	Mm
Precipitation of Warmest Quarter	bio18	Mm
Precipitation of Coldest Quarter	bio19	Mm

Appendix IV: Recommended and received medication

Recommended medication	n	Percentage (%)
Anti-allergies	91	10.1
Anti-inflammatory drugs	149	16.6
Painkillers	101	11.2
Robb ointment	78	8.7
Dettol	60	6.7
Others	421	46.8

Appendix V: Pesticides used by respondents for bedbug control

Pesticide trade name	Active ingredient	WHO class[a]	Frequency	Respondents' application (%)
Sevin dududust	Carbaryl 7.5%	II	230	25.57
Green leaf	Fipronil	II	142	15.73
Marathion II	Imidacloprid 21.4%	II	108	12
Diazinone	O,O-diethyl- O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate.		70	7.8
Dortor Doom	6% pyrethrin	II	68	7.56
Gladiator	Chlorpyrifos 480g/l	II	64	7.08
kungunil	Imidacloprid 200g/l	II	39	4.32
lava	Dichlorvos 1000g/L	Ib	39	4.20
Flavor	Unspecified	N/A	26	2.88
Dursban	Chlorpyrifos	II	25	2.76
Ectomin	cypermethrin high-cis 100g/l	II	19	2.16
Montem Doom	Allethrin (2.09 g/kg) and Resmethrin (0.39 g/kg)	II	15	1.68
Duduthrin	Lambda-cyhalothrin 17.5 g/lt.	II	13	1.44
Ortho	Bifenthrin 0.05%, Zeta-Cypermethrin 0.01%	II	9	0.96
Bedlam	Acetamiprid 200g/l	II	8	0.84
Dudu kwisha	Unspecified	N/A	8	0.84
Nozzle	Unspecified	N/A	5	0.60
Loyalty	Imidacloprid 700 g/kg	II	4	0.48
Promax	Propoxur 20% W/V	II	3	0.36
Ricatrix	Unspecified	N/A	3	0.36
Triatix	Amitraz	II	3	0.36

[a]WHO classification: Ia = Extremely hazardous; Ib = Highly hazardous; II = Moderately hazardous; III = slightly hazardous; U = Unlikely to present acute hazard in normal use; FM = Fumigant, not classified; O = Obsolete as a pesticide, not classified.

Appendix VI: Alpha diversity statistics for the bacterial metagenomes of bedbugs collected in nine counties of Kenya

County	Evenness	Richness	Shannon
Bomet (Lec.)	1.000000	4	1.39
Kericho (Lec)	0.27208397	227	1.48
Kiambu (Hemi)	0.46511	26	1.52
Kisumu (Hemi)	0.21071076	143	1.05
Machakos (Hemi)	0.44106611	54	1.78
Machakos (Lec)	0.30800598	26	1.00
Makueni (Hemi)	0.16216304	134	0.79
Mombasa (Hemi)	0.22499690	118	1.07
Nairobi (Hemi)	0.33901	138	1.67
Narok (Hemi)	0.086055	161	0.44
Narok (Lec)	0.25792943	212	1.38

Appendix VII: Inter-population beta diversity (%) in the metagenomes of bedbugs from different counties of Kenya, as estimated using Bray Curtis dissimilarity index

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	Kericho (Lec)	Mombasa (Hemi)	Kisumu (Hemi)	Nairobi (Hemi)	Bomet (Lec)	Narok (Lec)	Narok (Hemi)	Machakos (Lec)	Machakos (Hemi)	Makueni (Hemi)	Kiambu (Hemi)
Kericho (Lec)	0.00%										
Mombasa (Hemi)	62.76%	0.00%									
Kisumu (Hemi)	49.85%	29.30%	0.00%								
Nairobi (Hemi)	66.60%	19.42%	33.52%	0.00%							
Bomet (Lec)	99.89%	99.43%	99.61%	99.33%	0.00%						
Narok (Lec)	11.21%	65.78%	53.70%	69.08%	99.82%	0.00%					
Narok (Hemi)	22.95%	64.60%	51.96%	69.35%	99.81%	18.66%	0.00%				
Machakos (Lec)	94.98%	80.36%	87.88%	82.08%	93.64%	95.26%	95.01%	0.00%			
Machakos (Hemi)	92.47%	71.41%	82.14%	74.11%	95.26%	93.26%	93.21%	24.37%	0.00%		
Makueni (Hemi)	42.07%	30.93%	14.13%	39.73%	99.56%	46.20%	44.23%	88.35%	82.91%	0.00%	
Kiambu(Hemi)	96.39%	85.42%	91.12%	86.59%	90.93%	96.54%	96.38%	36.58%	39.04%	91.17%	0.00%

Appendix VIII: The relative abundance of pathogenic bacteria genera in all bedbug samples collected across Kenyan counties.

Genus	Percentage (%) abundance
Bacillus	0.09
Bartonella	0.15
Bordetella	0.01
Campylobacter	0.02
Clostridium	0.01
Corynebacterium	0.01
Enterococcus	0.12
Escherichia	3.31
Francisella	0.01
Listeria	0.04
Mycoplasma	0.02
Neisseria	0.01
Pseudomonas	0.38
Rickettsia	0.36
Salmonella	0.72
Shigella	0.01
Staphylococcus	0.18
Streptococcus	0.01
Vibrio	0.44
Yersinia	0.45