EFFICACY EVALUATION OF MOHLOLO INSECTICIDE BAIT ON GERMAN COCKROACH, *BLATTELLA GERMANICA* L

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[redacted]

MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE IN AGRICULTURE (PLANT PROTECTION), IN THE DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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DEDICATION

To my beloved family who I have always wanted to be my family.

ACKNOWLEDGEMENTS

I gratefully acknowledge:

- My supervisors team, Dr M. S. Mphosi and Professor P. W. Mashela, for all their guidance, support and advices in directing the progress and development of this mini-dissertation from the designing of the experiments through compilation of the actual research report. I am thankful to them for their insightful comments and constructive criticism at different stages of my research as well as their valuable inputs and the trust they showed on me during my undertaking of the research work.

- My parents, Mr Ketson Mbowane and Mrs Asnath Mbowane, as well as my sisters (Edith, Yvonne, Lullu and Maddy), brothers (Pilot and Isaya) and nephews (Petunia, Rivombo, Langutelani and Vutivi) for all their emotional and financial support. Without their unconditional support, this study would not have had a desirable conclusion. The National Department of Agriculture, Forestry and Fisheries for financial support that made my studies possible at the University of Limpopo.

- Limpopo Agro-Food Technology Station (LATS), for all the technical assistance that I received during the trials.

- The participating Mankweng and the University communities for allowing me access into their residential areas to collect data for this project.

- All the staff and students in the Department of Soil Science, Plant Production and Agricultural Engineering for their support — one way or the other.
- The Lord Almighty God for providing me with strength, wisdom, knowledge and understanding during my MSc study. May His Holy Name be glorified.

DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Plant Protection) has not previously been submitted by me or anybody for a degree at this or any other university; that it is my work in design and in execution and that all materials contained herein had been duly acknowledged.

Candidate: Clarah Mbowane ______________ Signature ______________ Date

Supervisor: Dr M.S. Mphosi ______________ Signature ______________ Date

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ABSTRACT

In most cases the discipline plant protection is restricted to the protection of propagules, the plants and their produce in storage, while excluding the protection of processed (cooked) food from contaminants. Generally, the German cockroach (*Blattella germanica* L.) is a serious contaminant of processed food and had recently been viewed as a pest worthy of control in plant protection when viewing the entire value chain of agricultural commodities. Due to the nature of the pest, it is quite difficult to control using registered agrochemicals on the market. An entrepreneur in the rural areas of Limpopo Province developed bait for cockroach control, which was code named Mohlolo during efficacy trials. The use of baiting in cockroach management is currently the sought after approach in the world where cockroaches are problematic. Bait has the advantage over liquid or dust formulations because baiting requires shorter service, has shown increased efficacy, and has reduced environmental contamination. The objectives of this study were to determine whether the effect of MIB would be age related in the suppression of populations of *B. germanica* under laboratory conditions, and also to investigate whether MIB would reduce populations of *B. germanica* in residential areas. Five concurrent *B. germanica* age-related experiments were run in a complete randomized design. 1-d, 2-d, 3-d, 4-d, 5-d old nymphs and adult German cockroach experiments were conducted at Limpopo Agro-Food Technology Station (LATS) laboratory, (53’21,41” S/29 23^{o} 44’19,95”E) hosted at the University of Limpopo. Each age group consisted of 10 nymphs and was put in 250 cm^{3} container that contained

Mohlolo insecticide bait (MIB). One gram (1 g) of Mohlolo insecticide bait and moistened cotton wick were put at the bottom centre of the 250 cm^3 containers containing nymphs based according to their respective ages. Residential experiments were conducted at UL student apartments. Two Bait containers were then placed in the kitchen, lodge, bathroom and bedroom. In the kitchen MIB containers were put behind the cooking stove and refrigerator, in the lodge the bait was placed next to the study table (because most study tables are also used during the eating time). In the bathroom the baits were put next to toilet seat and lastly in the bedroom, the bait was placed next to study table. According to the results, 100% mortality of 1-, 2-, 3-, 4-, 5-days nymphs and adult *B. germanica* was obtained after 7th, 2nd 3rd 3rd and 4th day, whereas LT50 values ranged from half a day to one and half (1.5) day. The study also demonstrated that *B. germanica* populations had high LT50 and LT100 values in residential areas compared to LT50 and LT100 values obtained in the laboratory experiments. This is probably due to the fact that residential areas that have low levels of sanitation availed the cockroaches with an opportunity to choose from many foods hence, it reduced the probability for cockroaches to feed on Mohlolo insecticide bait in the respective apartments. Results of this study showed that, Mohlolo bait toxicity and effectiveness as a control agent can reduce infestation of cockroaches in all developmental stages when applied at small quantity. Providing results of this study would provide information on resistance and tolerance of *B. germanica* nymphs and adult population against MIB in the laboratory and residential areas. It would also provide biological information on the efficacy of MIB against the German cockroaches.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Established German cockroach (*Blattella germanica* L.) infestations are difficult to manage along various components of the value chain of agricultural commodities in storage, restaurants and residential areas due to their behavioural and physiological adaptability (Owens and Bennett, 1982; Rust and Reierson, 1991). The German cockroaches have high reproductive potential (Owens and Bennett, 1982; Appel, 1992). The nymphs have a high survival rate because females carry the egg capsules during the entire time that the embryos are developing. This results in nymphs avoiding most hazards in the environment (Cornwell, 1976; Schal *et al*., 1997; Vatandoost and Mousavi, 2001).

Cockroaches are food and residential pests worldwide and infest places where food is stored, prepared, or served (Brenner, 1995). Cockroaches are well known resilient pests that are also difficult to manage (Goddard, 2003) and are proven carriers of pathogenic organisms that cause diarrhoea, dysentery, cholera, leprosy, plague, typhoid fever and viral diseases such as poliomyelitis (Tungtrongchitr *et al*., 2004). Furthermore, they carry eggs of parasitic worms and may cause allergic reactions including dermatitis, itching, swelling of the eyelids and more serious respiratory conditions. Roth and Willis (1957) reported that cockroach allergens stimulate asthmatic reaction in many asthmatics.

Insecticides (organophosphate, carbamates, oxy-carbamates and pyrethroids) had been used in the management of cockroaches. Nevertheless, frequent uses of insecticides in these classes over time led to the development of insecticide resistance (Batth, 1977; Cochran, 1989; Rust and Reierson, 1991; Lee *et al*., 1996). Insecticide resistance is the inherited ability of *B. germanica* species to survive exposure to a concentration of insecticide that is generally lethal to susceptible insects (Koehler *et al*., 1991; Valles *et al*., 1996).

Due to environmental and health issues, the Environmental Protection Agency (EPA) cancelled the use of most synthetic insecticides (Reid *et al*., 1990). This has emphasised the need for the development of environmental-friendly insecticides which can provide novel modes of action against insects' pest species and reduce the risks of gaining resistance to cross-resistance (Isman, 2006; Isman, 2008).

Worldwide, about 3 500 species of cockroaches have been reported (Roth and Willis, 1960). Only four species have pest status because they have adapted to living in buildings. The most common species in South Africa are the German cockroaches, *Blattella germanica* L. (Cornwell, 1968; Ross and Mullins, 1995), brown-banded, *Supella longipalpa* F. (Cornwell, 1968; Atkinson *et al*., 1990; Barcay, 2004), Oriental, *Blatta orientalis* L. (Blatchley, 1920; Cornwell, 1968) and American cockroaches, *Periplaneta americana* L. (Harker, 1956; Harker, 1960; Bell, 1984). However, the German cockroaches have been reported to cause health-related problems, with the ability to contaminate agricultural products along the value chains (Brenner, 1995).

High populations of these species can be of great concern when they occur in hotels, hospitals, restaurants and agro-food processing where they can contaminate food leading to considerable economic losses (Roth and Willies, 1957; Burges *et al*., 1974; Burges, 1979;).

German cockroaches usually prefer a moist environment with a relatively high degree of warmth (Cornwell, 1968; Baker, 1981). Thus, infestations are often at their worse during late summer months and mostly in warmer places like kitchens, laundries, warehouses and sewers. Most *B*. *germanica* are omnivorous insects that feed on a wide variety of foods, especially wherever prepared or served.

Cockroaches are best controlled through an integrated pest management (IPM) process of inspection, sanitation, exclusion and the use of low-toxicity insecticides. IPM is a decision-making process that involves regular monitoring and record keeping to determine if and when treatments are needed to prevent or solve pest problem. In Africa, cockroach control poses an increasing challenge for many pest controllers under the competing pressures of modern hygiene standards and economic necessities, without talking about the great adaptability of cockroach populations. Cockroach control study of 2006 and 2008 highlights continuing problems from all cockroach species in a wide variety of sectors.

It was reported that the current cockroach problems in domestic housing reported by pest controllers is 64% and 59% in hotels and restaurants, respectively, while 38% was in food retailing premises. Food processing premises are identified as problematic by 34%, while a significant number are treating schools, colleges and hospitals. In addition they have to deal with greater safety concerns, tackle bigger infestations and use longer baiting periods.

1.2 Problem statement

The control of *B. germanica* is still a big challenge in South Africa. The development of environmentally safe control strategies such as the use of safe insecticide baits against *B. germanica* population in the agro processing sector and household cross contamination in South Africa is inevitable. The researcher proposes to evaluate the efficacy of Mohlolo insecticide bait on *B*. *germanica* population and secondly, assess the effects of Mohlolo insecticide bait on age-Cohorts of *B. germanica* nymphs under controlled conditions.

1.3 Motivation

Mohlolo insecticide bait (MIB) is an insecticide which is being developed by a South African SMME in rural areas. In bait form, Mohlolo insecticide bait could be environment-friendly to the end-users who are being protected from the nuisance of cockroaches. In general, after application baits do not constitute a health hazard to food, pets or people.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study was to evaluate the efficacy of Mohlolo insecticide bait on *B*. *germanica* populations.

1.4.2 Objectives

Objective 1: To determine whether the effect of Mohlolo insecticide bait would be age-related on suppression of populations of *B. germanica* under laboratory conditions.

Objective 2: To investigate whether Mohlolo insecticide bait would reduce populations of *B. germanica* in residential areas.

1.5 Format of mini-dissertation

Following this General Introduction, a Chapter on Literature Review ensues; with each of the two subsequent chapters addressing each objective in sequence (Chapter 3-4). Findings in all chapters would then be summarised and integrated through providing the significance of the findings, recommendations with respect to future research and the conclusions in the final chapter.

1.6 Significance of the study

This study on the efficacy of Mohlolo insecticide bait on cockroaches could be an additional contribution in the field of biology because it will offer an alternative control for costly prepared insecticide and make use of bait that is prepared by locals in the Province. This bait would also help people living in remote areas that have no financial access to purchase commercial insecticide in pesticide stores. The procedure or processes that would be explained in this study would be encouraged of a new product from Mohlolo insecticide bait that is easy to administer and cost effective, and when properly prescribed and used have advantage of being relatively free of side effects.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Due to an increase in cockroach resistance incidences resulting from frequent use of synthetic insecticides, increasing cockroach population densities are inevitable (Cochran, 1995). Successful cockroach control strategies have been reported under control programmes which used long-lasting insecticides and/or indoor residual spraying. The disadvantage with the former cockroach programmes is that they were environmental-unfriendly (Miller, 2004).

Since insecticides like bait, dust and sprays used to manage cockroaches vary in cost, formation, toxicity and effectiveness; they also have different negative effects to the environment (McLaughlin, 1991). The active ingredients in the majority of the insecticide sprays and foggers used for controlling cockroaches are mostly synthetic pyrethroids with active ingredients that include allectin, deltamethrin and cypermethrin (Appel, 1990; Ross, 1993). However, insecticides like pyrethroids may pose both short and long-term health risks to humans (Koehler *et al*., 1991; Leng *et al*., 2003). Children are at higher risks of pesticide poisoning due to their behaviour and developmental stage (Koehler *et al*., 1991). In addition to the pesticide active ingredients, adjuvant such as piperonylbutoxide which is used to enhance the knock down effect of pyrethroid and their inert ingredients may also cause health problems for sensitive individuals such as children, older adults and people with chronic illnesses (Roth and Willis, 1957; Pai *et al*., 2005).

2.2 Biology

German cockroaches, *Blattella germanica* (L.) are hemimetabolous (Rence and Loher, 1975), which means that they are only having three life stages: the egg, larva and adult. Nymphs are dark brown in color with a large tan spot on their protonum. They are approximately 1.5 mm as newly emerged, while adults are approximately 1.6cm long. Adults are light brown in colour and have two dark stripes on their pronotum extending longitudinally down the body under the wings. The male has a tapered abdomen while the female has rounded abdomen (Ebeling *et al*., 1974). Although both sexes possess wings, they are not able to fly. When the adult females become sexually active, they often mate multiple times, although one mating is usually sufficient to fertilize all their eggs (Schal *et al*., 1997). They are oviparous which implies that offsprings are born. However they carry their ootheca until just prior to hatching (Schal *et al*., 1997). The gravid cycle is approximately 21-28 days (Schal *et al*., 1997). The female produces 30-40 eggs per oothecal case (Willis *et al*., 1958) and can produce 4-6 broods in its life-time (Schal *et al*., 1997).

2.3 Health effects to humans

German cockroaches have a world-wide distribution and in addition to domiciles, can be found in restaurants (Rust and Reierson, 1991), hospitals (Kitae *et al*., 1995; Elgderi *et al*., 2006) and even aboard naval vessels. They are usually found in kitchens, bathrooms or other areas where water is readily available. They are nocturnal scavengers capable of living off human waste foodstuffs (i.e. crumbs, residues on dishware, *etc*). During the day, they usually remain sequestered in harborages.

German cockroaches commonly carry potentially pathogenic bacteria such as *Klebsiella*, *Enterobacter*, *Serralia* and *Streptococcus* (Kitae *et al*., 1995; Elgderi *et al*., 2006). No direct transmission of disease to humans has been demonstrated; however, cockroaches have potential of transmitting these pathogens via contamination of food preparation surfaces and utensils (Kitae *et al*., 1995). New data has found that a great number of pathogens isolated from wild strain cockroaches are multiple antibiotic resistant (Elgderi *et al*., 2006). Dust from cockroach droppings aggrevates asthma (Roth and Willis, 1957).

2.4 Chemical control

The oldest group of chemicals used to control cockroaches is inorganic compound such as boric acid and sodium fluoride, but these were slow acting powders that worked both via contact and orally when ingested during grooming (Ebeling *et al*., 1974). Because these were designated for a variety of insects, these dusts were effective on larger cockroaches (*Periplaneta*) and offered no real control of the German cockroaches. Chlorinated-hydrocarbons such as DDT and chlordane were the next group used to control the German cockroaches. These classes of chemicals were effective with long-lasting residual sprays and acted on the sodium channel causing repetitive firing (Grayson, 1964). However, within 10 years of usage, the German cockroach developed physiological resistance to chlorinated- hydrocarbons and chlordane which spread throughout the world. Due to environmental concerns, the Environmental Protection Agency (EPA) cancelled the use of chlorinated-hydrocarbons in the late 1970s (Reid *et al*., 1990).

2.5 Organophosphates

The organophosphates (OP) are derivatives of phosphoric or phosphonic acid (Zhu and Clark, 1995). There are approximately 40 OP and as a group they account for approximately half of the insecticide used. The OP is often used in and around homes to control cockroaches, termites, lawn insects, ants, cockroaches, fleas, ticks, and mosquitoes. However, the majority of the chemical is used on food crops. Examples of OP pesticides include chlorpyrifos, azinphos methyl, methyl parathion and phosmet.

The OP kills insects and other animals by impacting the function of the central and peripheral nervous system (Zhu and Clark, 1995; Bryne and Toscano, 2001). They inhibit the activity of acetylcholinesterase (AChE), an enzyme which breaks down the neurotransmitter acetylcholine. The resulting interference with nerve transmissions is of such a magnitude that it actually kills insects. When overdosed, OP can also kill people and pets. This increasing concerns however, led to studies in animals that show that even a single, low-level exposure to certain OP during particular times of early brain development can cause permanent changes in brain chemistry as well as changes in behavior, like hyperactivity. Other research also suggests that early childhood exposures to pesticides, can go undetected because of the lack of overt symptoms to certain organophosphates, and can lead to lasting effects on learning, attention, and behavior (Bryne and Toscano, 2001).

2.6 Carbamates

Investigations of chemicals that exert an anticholinesterase action on the nervous system similar to organophosphates led in the 1950s to the development of the carbamate insecticides. Carbamate insecticides are derivatives of carbamic acid, HOC (O) NH2. They have the general formula shown below where R is an alcohol, oxime or phenol and R1 is hydrogen or a methyl group.

O H
|| |
R-O-C-N-R1

Carbamates have approximately 25 compounds that are in use today. However, the most common compounds are four and vary in the spectrum of activity, mammalian toxicity and persistence. They are relatively unstable compounds that break down in the environment within weeks or months (Robert, 2002; Takino *et al*., 2004). Carbamates are commonly used as surface sprays or baits in the control of household pests. Carbaryl, the first successful carbamate was introduced in 1956 and two distinct qualities have made it a widely used insecticide. First, it has very low mammalian, oral and dermal toxicity. Secondly, it has a rather broad spectrum of insect control. This has led to its wide use as a lawn and garden insecticide.

The second carbamate includes Propoxur (Acgih, 1986), which is highly effective against cockroaches that have developed resistance to organophosphates. It was commonly used by pest control operators for the control of cockroaches and other household insects in restaurants, kitchens and homes. Cabaryl and Propoxure are no longer recommended for use as cockroach control. The third carbamate is called Bendiocarb and has found its greatest use as a household, turf and ornamental insecticide. Methomyl is the last and often applied as adult fly bait. Several carbamates have systemic use in plants because they have a high water solubility which allows them to be taken up by the roots and into the leaves of plants.

The mode of action of carbamate insecticides is very similar to that of the organophosphate insecticides as they inhibit cholinesterase enzymes (Padilla, 2006). However, they differ in action from the organophosphate compounds in that the inhibitory effect on cholinesterase is brief. The reversal of the effect of carbamates is so rapid that measurements of blood cholinesterase levels in human beings or other animals exposed to carbamates are likely to be inaccurate and always in the direction of appearing to be normal (Saadeh and Farsakh, 1996). In insects, the effects of carbamates are primarily those of poisoning of the central nervous system where cholinergic reactions are thought to take place. However, the insect neuromuscular junction is not cholinergic, as it is in mammals (Padilla, 2006).

2.7 Oxycarbamates

Oxycarbamates were developed into commercial pesticides in the 1950s. It is a very huge family which members are effective as insecticides, herbicides, and fungicides, but they are most commonly used as insecticides. More than 50 carbamates are known. The most often used Insecticides basic and other applications contain 40 members of carbamate group and are: aldicarb, asulam, bendiocarb, carbaryl, carbetamid, carbofuran, carbosulfan, chlorpropham, desmedipham, ethiofencarb, formetanate, furatiocarb, fenoxycarb, isoprocarb, methiocarb and *etc*.

Like the Organophosphates, most oxycarbamates are active inhibitors of acetylcholinesteraze (ACHE), but some oxycarbamates as benzimidazole have no acetylcholinesterase activity. Oxycarbamates toxicity to insects, nematodes, and mammals is based on inhibition of ACHE. The ACHE is the enzyme responsible for the hydrolysis of acetycholine into choline and acetic acid. Acetylcholine (ACh) is a substance that transmits a nerve impulse from a nerve cell to a specific receptor such as another nerve cell or a muscle cell. Acetylcholine, in essence, acts as a chemical switch. When it is present (produced by nerve cell) it turns the nerve impulse on. When it is absent the nerve impulse is discontinued. The nerve transmission ends when the enzyme ACHE breaks down the acetylcholine into choline and acetic acid. Without the action of this enzyme acetylcholine builds up at the junction of nerve cell and the receptor site, and the nerve impulse continues. Oxycarbamate insecticides block or inhibit the ability of this enzyme ACHE to break down the acetylcholine and the nerve impulse (MacHemer and Pickel, 1994; Kamrin, 1997).

2.8 Pyrethroids

Pyrethroids became popular for cockroach control in the 1970s. Prior to this time, pyrethrin which is a natural pesticide derived from chrysanthemums in the Family Compositae had been used to augment inorganic pesticides. In the late 1950's, pyrethroids which is the synthetic analogue of pyrethrins became commercially available. This pyrethroid was called "Type I' pyretroid and were not photo stabile, and when fed to cockroaches many of them were able to metabolize the chemical and fully recover. Then the other one was called "Type II" pyrethroids and became popular in the 1970s. "Type II" were photo stabile and used in spray formulation, and had excellent residual effects against cockroaches. All pyrethroids acted on the sodium channel to interfere with the transmission of nerve impulses. As with all the other classes of chemical, German cockroaches later developed resistance to pyrethroids (Cochran, 1994). Pyrethroids are still used today; however, they are used as flushing agents to drive cockroaches out of their harborage (Fuchs, 1988). However, cockroach developed resistance to this chemical and the mechanisms involved in pyrethroid resistance include: decresed cuticular penetration, behavioral avoidance, and metabolic detoxification. Wuu *et al*. (1998); Valles *et al*. (2000) reported decreased rate of cuticular penetration as one of pyrethroid resistant mechanisms in German cockroaches.

2.9 Insect growth regulators

The safest chemicals reported are insects' growth regulators mainly the juvenile hormone analogs such as hydroprene and pyriproxifen (King and Bennett, 1989). These chemicals mimic insect juvenile hormone affecting the endocrine balance and causing developmental disturbances such as molting inhibition, morphogenetic abnormalities, longer developmental time and reproduction suppression (King and Bennett, 1989; Reid *et al*., 1994). However, juvenile analogs lack an ability to suppress a population quickly (Zeman, 1991; Koehler and Patterson, 1991). Generally, unless more than 80% of the population is strongly affected by the juvenile hormone analog, viable young nymphs can still be produced (Reid *et al*., 1994).

2.10 Insecticidal baits

The use of insecticidal baits is an effective control strategy for controlling *B. germanica* species to date (Bennet *et al*., 1997). Insecticidal baits provide several advantages over other insecticide application methods. Bait offers the advantage of low odour, little dispersion, stability and ease of application compared with aerosol and spray formulations (Appel, 2004). Majority of toxic baits are specific to the target organism, or at least provide greater selectivity than liquids and dusts. Also, baits greatly reduce problems with runoff and drift from liquid and dust insecticide formulations.

2.11 Management strategies

Management of cockroach populations in urban environments has shifted from predominant use of insecticide sprays to the inclusion of baits in management programmes (Reierson, 1995). The change had been facilitated by the general perception that baits were safer and appeal to public concern about risks associated with pesticides in the domestic environment. Moreover, baits fit well into integrated pest management (IPM) objectives of reducing pesticide usage while maintaining effective suppression of cockroach populations (Hamilton, 1990).

The efficacy of baits is determined by the collective performance of their components, including the active and inert ingredients, food base, odorants and design in the case of base housed within a container. Mohlolo insecticide bait was developed by a South African SMME and is mainly marketed through the third economy, where products are not registered with any authority. Mohlolo insecticide bait is mixed with bread as the attractant. Currently, no work has ever examined the levels of infestation of *B. germanica* populations in apartments or homes and their resistance to bait in South Africa.

2.12 Addressing the identified gaps

Bait Computer models based on life table data of German cockroaches have indicated that reductions of 95-99% are necessary to maintain populations at undetectable levels (Grothaus *et al*., 1981). The question is whether commonly used methods of *B. germanica* control in South Africa provide such suppression. Few studies had examined the levels of infestation of German cockroach populations in apartments or homes, while fewer have assessed the impact of traditional control measures on population levels. However, many non-chemical techniques have been demonstrated to give partial control, but no approach had been able to give adequate results to date.

2.13 Summary of the gaps to be investigated

Failure in controlling *B*. *germanica* nymphs can be attributed to insufficient data in age-related studies of *B*. *germanica* species. Resistance to chemical control needs understanding of physiological processes and biochemical pathways in relation to the active ingredients. There is still little information available on the relationships between the evolution of resistance mechanisms and the resultant resistance factors on *B*. *germanica* population. Failure in suppression of *B*. *germanica* population below economic threshold level still remains an issue in pest control programmes. There is no enough documented information on the control of cockroaches in dwelling places; hence our study will be the first official report on the use of bait in controlling *B*. *germanica* population in South Africa around residential areas.

CHAPTER 3
RESPONSES OF AGE- RELATED POPULATION OF BLATTELLA GERMANICA TO MOHLOLO INSECTICIDE BAITING UNDER LABORATORY CONDITIONS

3.1 Introduction

Heavy infestations of cockroaches (Dictyoptera: Blattelidae) can be effectively managed by chemical control measures such as the residual spray, aerosol, dust, baits and gels, followed by environmental management to deprive them of food and shelter (Cochran, 1995; Lee and Lee, 2000). Until recently, the management of cockroaches relied mostly on sprays based on synthetic insecticides (Cornwell, 1976). However, with the development of gel baits, which can be selectively applied where the cockroaches are living, the situation has now changed (Harbison *et al*., 2003). Baits have an advantage over synthetic insecticides and can be selectively used insensitive areas, such as premises used for the preparation of food, hospitals and preschools and can be applied or sealed in small areas where other insecticides can not be able to reach such as cracks and crevices (Appel, 1992; Appel and Tanley, 2000).

Mohlolo insecticide bait was developed by an SMME and is being used by locals to control German cockroach population. The active ingredient is still not revealed due to the incomplete IP filling process. The bait relies mainly upon bread quantity serving as an attractant to cockroaches. Despite its acceptance and use in the third economy, its efficacy is not yet documented. The objective of this study was to determine whether the effect of Mohlolo insecticide bait (MIB) would be age-related on suppression of populations of *B. germanica* under laboratory conditions.

3.2 Materials and methods

3.2.1 Study location

The study was conducted at Limpopo Agro-Food Technology Station (LATS) laboratory, (53’21,41” S/29 23^{o} 44’19,95”E) hosted at the University of Limpopo. The laboratory was maintained at 24 –28ºC, ambient humidity and a photoperiod of 10: 14 (L: D).

3.2.2 Experimental design and procedures

The German cockroaches were collected from University of Limpopo student residences and kept in a 5-L bucket. Cockroaches were allowed to mate and all gravid females were separated from males for hatching purposes and were kept in 250 cm^3 container. Female cockroaches that had dropped the ootheca were removed from the containers. Females that did not drop their oothecae were allowed to remain in the container until hatching was completed. Each dropped ootheca was kept in the container until eggs had hatched. After hatching, 10 newly emerged nymphs were transferred into a clean (18.7 x 13.3 x 9.5 cm) container containing soaked cotton wick at the bottom as source of water for the nymphs. The containers were greased with petroleum jelly on the upper inner portion to prevent nymphs from escaping.

Five concurrent *B. germanica* age-related experiments were run in a completely randomised design, with 10 replications. The 1-, 2-, 3-, 4- and 5-day old German cockroach nymphs experiments were conducted under conditions previously described (3.2.1.) Each age group consisted of 10 nymphs and was put in 250 cm^3 container that contained MIB. One gram (1 g) of Mohlolo insecticide bait and moistened cotton wick were put at the bottom center of the

250 cm^3 containers with nymphs classed according to their respective ages. All age groups were exposed to MIB for ten days in order to determine lethal time to mortality (LT100%). Days to 50% and 100% mortality were correlated against the mean mortality percentage (Silverman *et al.*, 1991). The control consisted of untreated bread as food and cotton wick as source of water.

3.2.3 Data collection

The number of nymphs that died was recorded daily and was expressed as mortality percentage.

3.2.4 Data analysis

Lethal time (LT_{100}) values were generated by PROC UNIVARIATE and analysed by analysis of variance (ANOVA) (PROC GLM) in SAS 8.2 software (SAS Institute 2001). Cockroach stage-related mortalities with significant ($P \leq 0.05$) treatment means were subjected to lines of the best fit using mortality responses to increasing times. The generated relationships were modeled by the regression curves estimates from the quadratic equation ($Y = b_2x^2 + b_1x + a$), where y = mortality response and X is the optimum exposure time, which was derived from $x = -b_1/2b_2$.

3.3 Results

The mortality of the age-related stages of cockroaches was significantly affected by the period of exposure to MIB (Appendix 3.1-3.5). The period of exposure contributed 97, 100, 100, 100, 100 and 98 of the total treatment variation of age-related mortalities.

In all stages, when mortality (y-axis) was regressed over increasing period of exposure (x-axis), the relation had density-dependent growth patterns, which were characterized by quadratic curves (Figure 3.1). In 1-, 2-, 3-, 4- and 5-day old nymphs, the quadratic equation models explained 97%, 100%, 100%, 100% and 100%.and age-related mortalities, respectively.

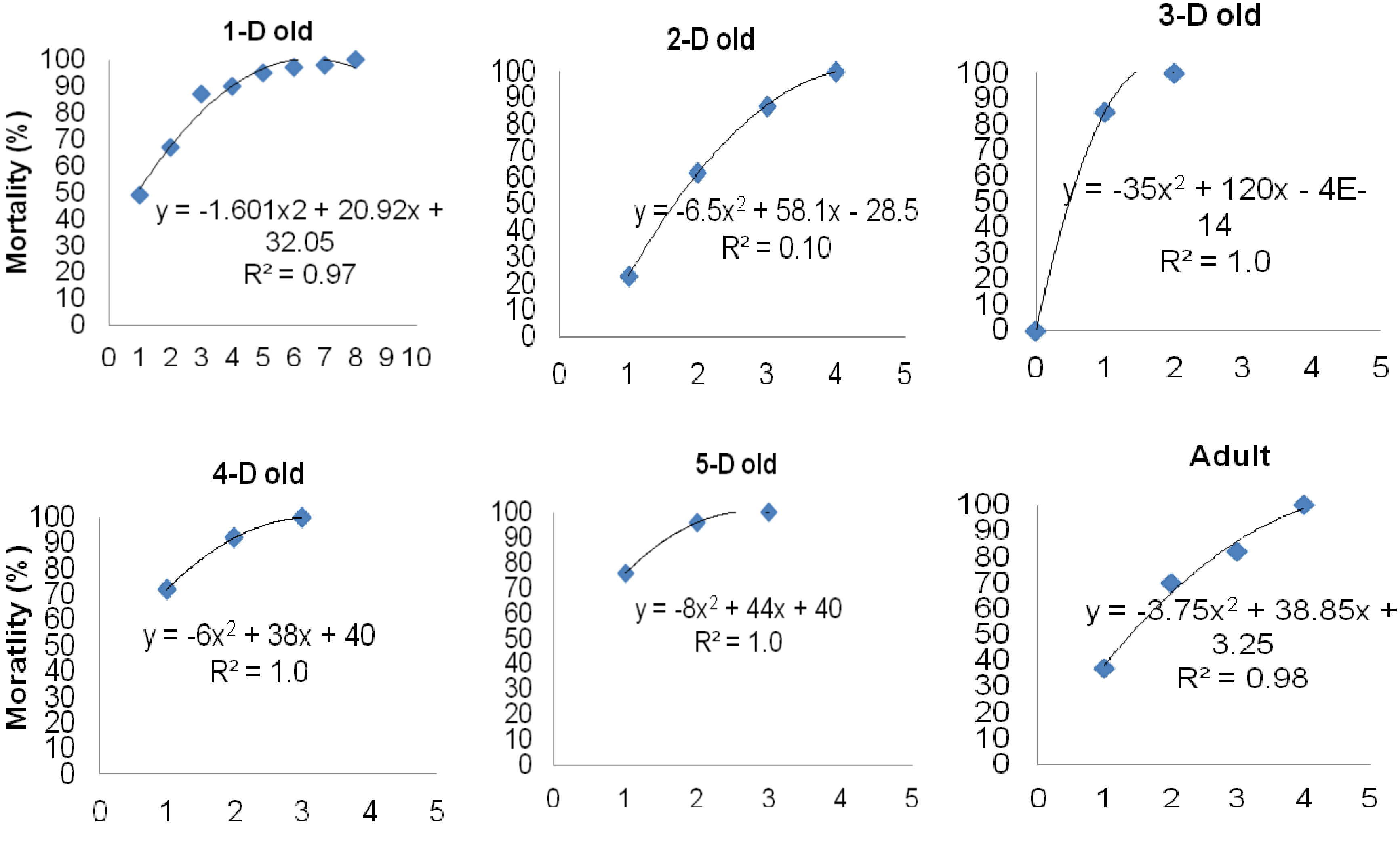

Figure 3.1: The relationship between mortality of 1-, 2-, 3-, 4-, 5 days nymphs and adult *Blattella germanica* and period of exposure to MIB.

Using the $x = -b_1/2b_2$ relationship in the quadratic equations, the optimum period of exposure (x) to achieve optimum mortality in 1-, 2-, 3-, 4- and 5-day old nymphs had a declining trend (Table 3.1), which agreed with LT_{100} values (Table 3.2) which were directly derived from the quadratic curves.

Table 3.1: The optimum period of exposure (x) to achieve optimum mortality in 1-, 2-, 3-, 4-, 5-day old nymphs and adult *B. germanica*.

Age	Quadratic equations	R^2	X	Y
1-d old	$Y = -1.601x^2 + 20.92x + 32.05$	0.97	6.54	128
2-d old	$Y = -6.5x^2 + 58.1x - 28.5$	0.10	4.47	101
3-d old	$Y= -35x^2 + 120x$	1.0	1.71	103
4-d old	$Y= -6x^2 + 38x + 40$	1.0	3.20	100
5-d old	$Y= -8x^2 + 44x + 40$	1.0	2.75	101
Adults	$Y = -3.75x^2 + 38.85x + 3.25$	0.98	5.18	104

$$x = -b_1/2b_2$$

Table 3.2. Optimum exposure days to Mohlolo insecticide bait to reach maximum mean mortality in *B*. *germanica*.

Age	**LT50**	**LT100**
1-d old	1.0	7
2-d old	1.5	4
3-d old	0.5	2
4-d old	0.5	3
5-d old	0.5	3
Adults	1.5	4

Fitting the best line between the optimum time of exposure (y-axis) over the age-related nymphs (x-axis) resulted in relations (Figure 3.2). The linear quadratic relation explained 97%, 100%, 100%, 100% and 100% variation in optimum exposure period was due to age-related nymphs.

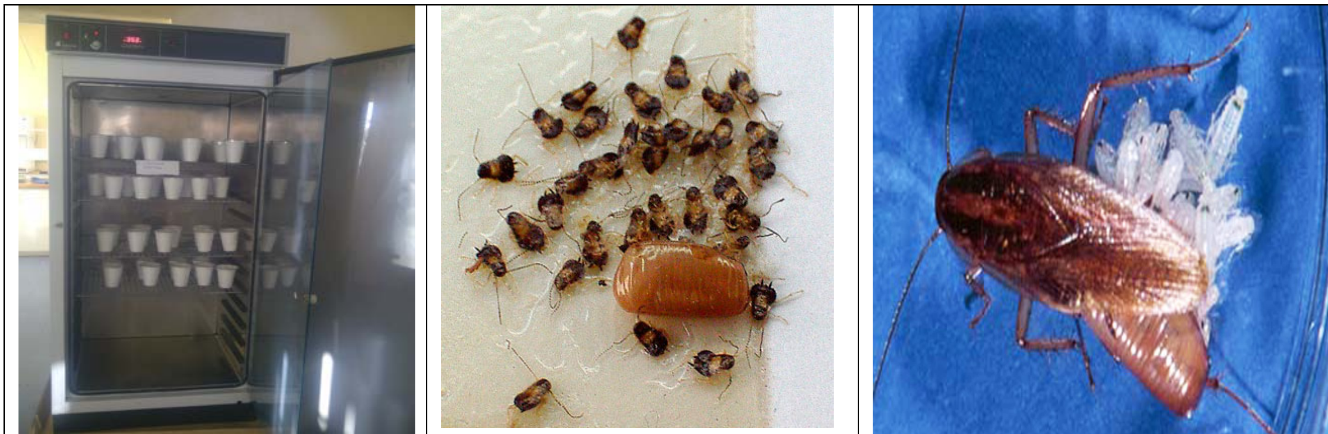

Figure 3.2: Laboratory experiment of 1-, 2-, 3-, 4-, 5-old nymphs and adult *Blattella germanica* contained per container in the presence of MIB.

3.4 Discussion

Mortalities of the age-related nymphs were significantly affected by the exposure time, which agreed with the concepts of dosage and dose. Dosage is the amount that should be placed in the environment, while dose is the amount of pesticide to be taken by the pest to effect behavioural changes (Altshuler, 1981). In this study, dosage at 1 g MIB remained constant since the product was applied once. Thus, the observed significant effect of the period of exposure of age-related nymphs of cockroaches could be attributed to dose.

Pests have different mechanisms to render dose ineffective, with the common ones in stomach poisons being detoxification through metabolism and then excretion from the organism (Mathews, 1980; Scott, 1990; Soderlund and Bloomquist, 1980). The detoxification mechanism reduces the concentration of the pesticide to below the level which is required to effect the desired result, which in this case is to kill.

The active ingredients of MIB are not known. However, organophosphates and carbamates constitute two major commercially available stomach poisons used in pesticides. The two groups have different mechanisms of bioactivity in insects. Organophosphates act on phosphorylation of the active sites in an irreversible action (Evans, 1973; Nelmes, 1971). In contrast, the carbamates act by carbomolytion of the active site, which is a reversible action (Evans, 1975). In all nymph stages, the effect of MIB was permanent, suggesting that the active ingredient of MIB could probably constitute an organophosphate.

Mortalities of all nymph stages and adults against exposure time were characterised by density-dependent mortality (DDM) patterns or time-mortality responses (Cochran, 1989), which are important features in biological entities which encounter stressful environmental conditions (Salisbury and Ross, 1992). The DDM patterns are characterised by three responses: stimulated response, saturated (neutral) response and inhibited response. Quantitatively the DDM patterns are characterised by the quadratic curves (Liu *et al*., 2003), which were consistent in all tested age-related stages of cockroaches.

In the DDM pattern, mortality was optimized through $x = -b_1/2b_2$ (Mamphiswana *et al*., 2010), where delayed DDM suggested tolerance tendency of the nymph stage to MIB. The observed high tolerance tendencies in 1-day old nymphs agreed with observations of others in cockroach studies (Koehler *et al*., 1993; Qian *et al*., 2010). Specifically, Koehler *et al*. (1993) observed tolerance tendencies on *B. germanica* during the early stages of nymphs exposed to bendiocarb, cypermethrin and chlorpyrifos insecticides when compared with adults. Incidentally, the three insecticides are the organophosphates.

High tolerance tendencies of early stages in nymphs of *B. germanica* had been attributed to acety-cholinestarase (ACHE) inhibition in the nerve tissues (Casida, 1955). ACHE is a key enzyme in the transmission of nerve impulses, specifically in termination of cholinergic synaptic transmission in insect and mammals (Koehler *et al*., 1993). Ahmad *et al*. (2001) attributed early tolerences to insecticiodes in nymphs of cockroaches to an increased hydrolase activity (Shi *et al*., 2002).

The least resistant observed in adults exposed to MIB confirmed those observed in other studies. Koehler *et al*. (1993) observed that adult cockroaches had the highest susceptibility to organophosphates. The differences between nymphs and adults could be due to higher metabolic rates in nymphs which lead to faster excretion of the toxic substance from the body, while the opposite was true in adults (Koehler *et al*., 1993).

3.5 Conclusions

Density-dependent mortality (time-mortality) response patterns over exposure period to MIB were observed in age-related nymphs of cockroaches under controlled conditions. Early nymphs stages were highly resistant to MIB as demonstrated by delayed time-mortality responses of at least one week while late stages and adults were highly susceptible to the absence of reversal in the effects of MIB on various stages of cockroaches, it was probable that the active ingredient of MIB was on organophosphate.

CHAPTER 4
RESPONSES OF POPULATIONS OF BLATTELLA GERMANICA TO MOHLOLO INSECTICIDE BAITING IN RESIDENTIAL AREAS

4.1 Introduction

Management of the German cockroach *Blattella germanica* (L.) populations in residential environments has shifted from predominant use of insecticide sprays to the inclusion of baits in management programmes (Reierson, 1995). The change was facilitated by the general perception that baits were safer and therefore appeal to the public's concern about risks associated with pesticides in domestic environments. Moreover, baits fit well into integrated pest management (IPM) objectives of reducing pesticide usage while maintaining effective suppression of cockroach populations (Hamilton, 1990). The efficacy of baits is determined by the collective performance of their components, including the active and inert ingredients, food base, odourants and designs (Appel, 1990).

Unlike sprays, baits are either contained in a protective unit or are placed in areas close to the harborages and foraging areas. This highly specialised application process has the advantage of targeting the pest species, while simultaneously allowing for use of less chemical (Kopanic and Schal, 1997). Currently, there are many active ingredients employed in baits used for German cockroach control. However, baits are effective when they are palatable and the active ingredients do not deter feeding (Appel, 1990). Current active ingredients are toxic enough to deliver a lethal dose in one meal (Wang *et al*., 2004). However, they act slowly enough to allow time for the cockroach to return to their harbourage after feeding. The objective of this study was to investigate whether Mohlolo insecticide bait (MIB) would reduce populations of *B*. *germanica* in various environments within the residential areas.

4.2 Materials and methods

4.2.1 Study location

The study was conducted in four residential apartments at the University of Limpopo (53’21,41” S/29 23^{o} 44’19,95”E) in 2010. The specific areas included kitchens, lodges, bathrooms and bedrooms.

4.2.2 Experimental design and procedures

A completely randomised design (CRD) was used in the study with the four treatments. Approximately 1 g of MIB was placed in 6 cm^3 container, with 2 containers placed in the kitchen, lodge, bathroom and bedroom. In the kitchen MIB containers were placed behind the cooking stove and refrigerator, in the lodge next to the table which was used for eating and studying. In the bathroom the baits were placed behind the toilet seat, while in the bedroom they were placed next to the study table.

Figure 4.1: Population of *Blattella germanica* in residential area after exposure to Mohlolo insecticide bait.

Figure 4.2: Young *Blattella germanica* in residential area during nymphs feeding on poisoned dead adult cockroach, a process known as necrophagy.

4.2.3 Data collection

Dead cockroaches were collected daily from each experimental unit for 13 consecutive days. Data comprised all age-groups.

4.2.4 Data analysis

Lethal time (LT100) values were generated by PROC UNIVARIATE and analysed by analysis of variance (ANOVA) (PROC GLM) in SAS 8.2 (SAS Institute, 2001). Mean separation was done using the Tukey test at the probability level of 5%. Data were further expressed as percent mortality over exposure time, with optimum exposure time determined as described previously (chapter 3).

4.3 Results

In Apartment 1, 2 and 3, the treatments had highly significant effects on cockroach mortality and explained 45%, 50% and 16% total treatment variation in mortality, respectively (Table 4.1). Cockroach mortality over exposed time had density-dependent growth patterns, which were characterised by quadratic curves (Figure 4.1-4.9). At least 50% reduction in *B. germanica* numbers was achieved within 7 and 10 days after the cockroaches were exposed to the MIB, while 100% mortality was attained within 12 and 14 days (Table 4.2). Cockroach mortality over exposure time attributed 99.8%, 99.34%, 99.84%, 99.35%, 98.73%, 99.31%, 99.92%, 97.31%, 98.08% and 98.51% to the variation in mortality percentage in Apartment 1 K,1 L,1 BTR, 1 BDR, 2 K, 2 L, 2 BTR, K, 3 L, 3 BDR, respectively (Figure 4.1-4.9).

The optimum exposure time in all experimental units was negative, ranging from -0.54 to -32.01 (Table 4.2). Similarly, the optimum population of cockroaches ranged from 1 to -94 (Table 4.2). In contrast, LT50 ranged from 7 to 10 days, while LT100 ranged from 12 to 14 days (Table 4.3).

Table 4.1. Partitioning of Analysis of variance for *B. germanica* population

Mortality

Source	Apartment 1			Apartment 2			Apartment 3		
	Df	SS	%	Df	SS	%	Df	SS	%
Unit	3	27864.4	45.2**	2	6884.5	48.9**	2	5112.0	15.5**
Error	47	33781.6	54.8**	34	7190.2	51.1**	29	27820.9	84.5**
Total	50	61646.0	100	36	14074.7	100	41	32933.0	100

ns= not significant at 10%, ** = significant at 5% level of probability

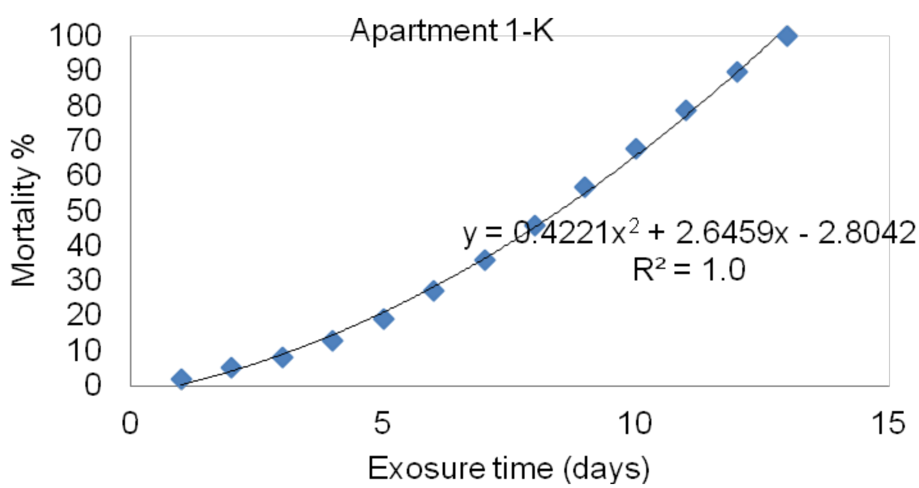

Figure 4.3. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 1-K after 14 days of exposure.

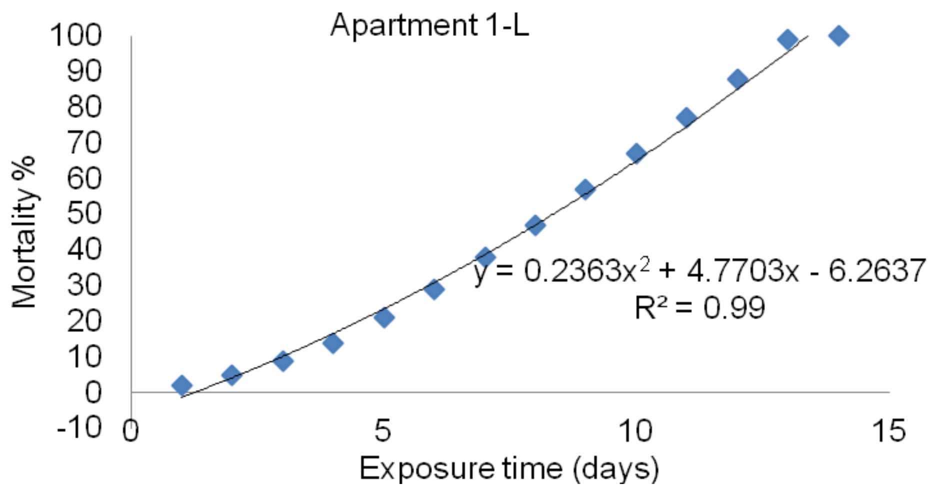

Figure 4.4. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 1-L after 14 days of exposure.

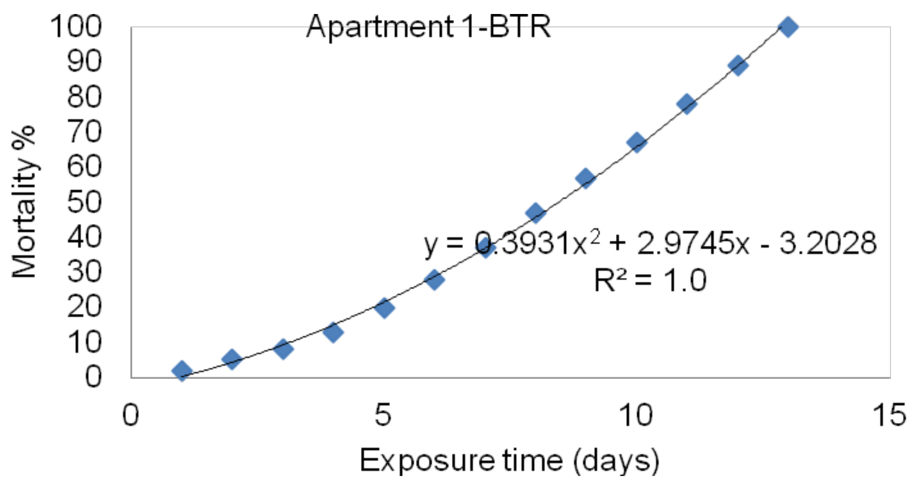

Figure 4.5. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 1-BTR after 14 days of exposure

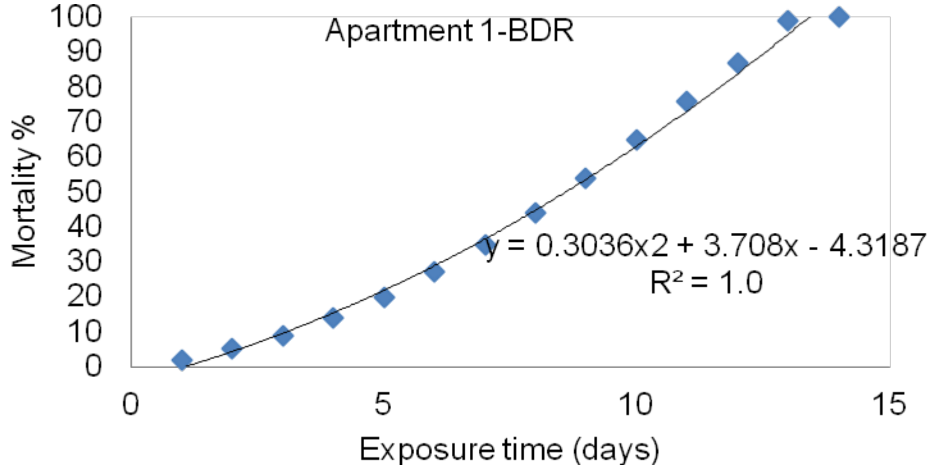

Figure 4.6. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 1-BDR after 14 days of exposure.

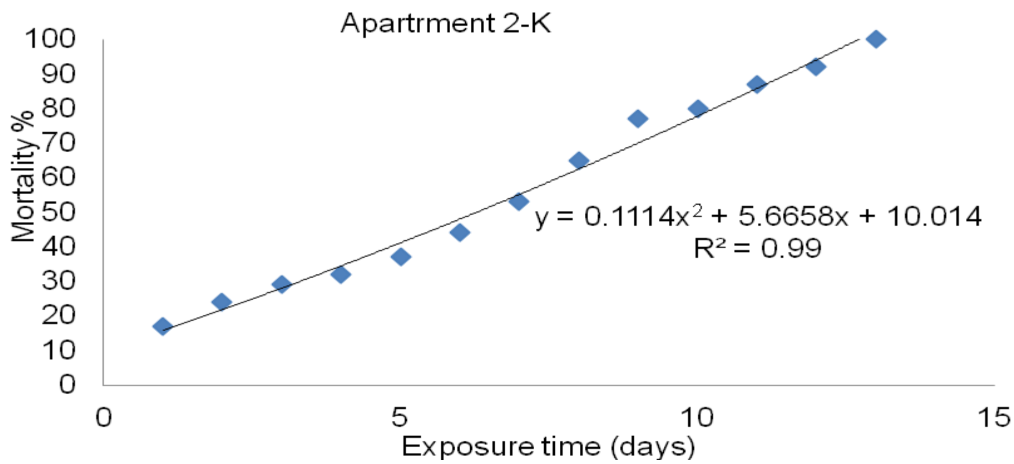

Figure 4.7. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 2-K after 14 days of exposure.

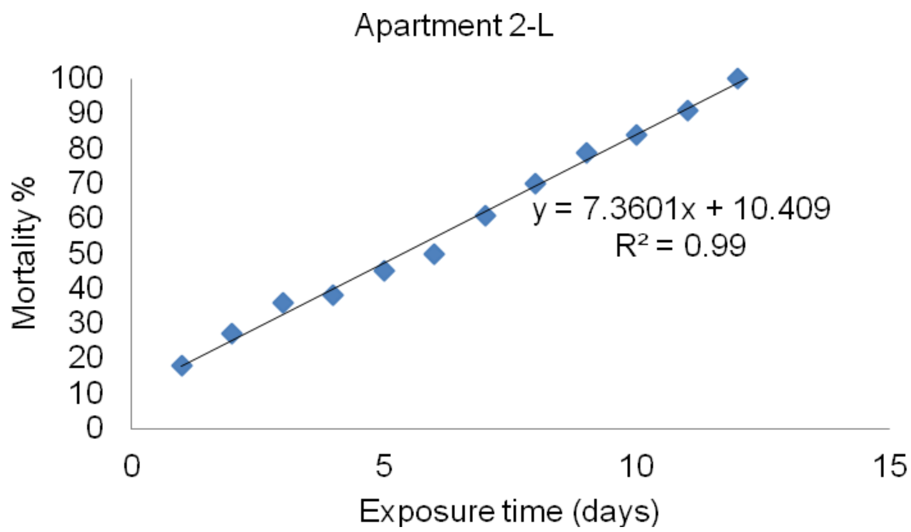

Figure 4.8. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 2-L after 14 days of exposure.

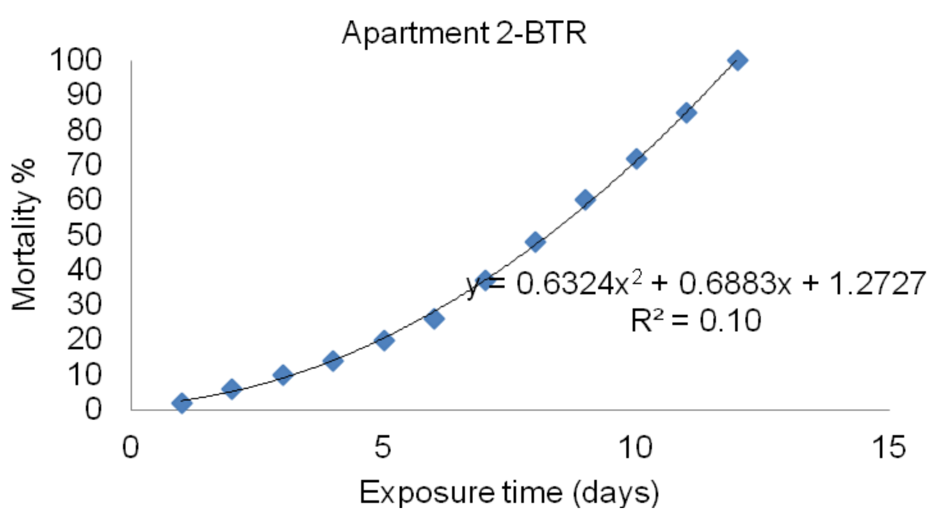

Figure 4.9 Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 2-BTR after 14 days of exposure.

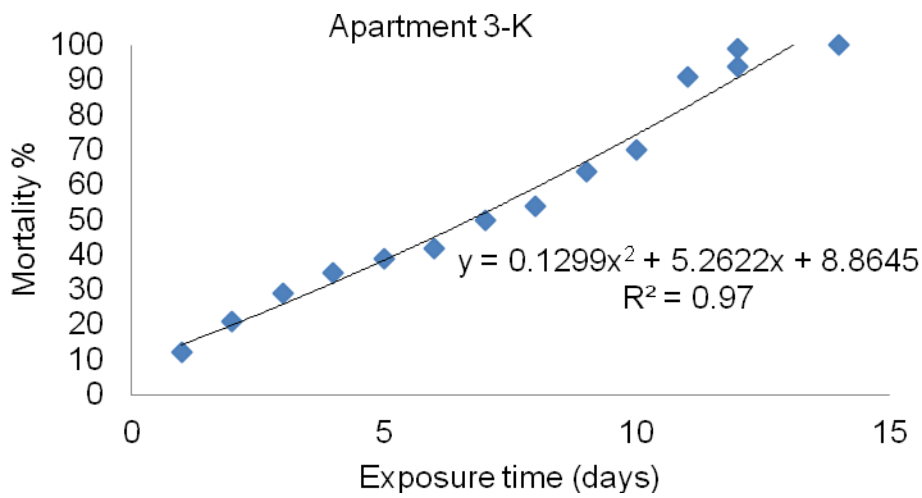

Figure 4.10. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 3-K after 14 days of exposure.

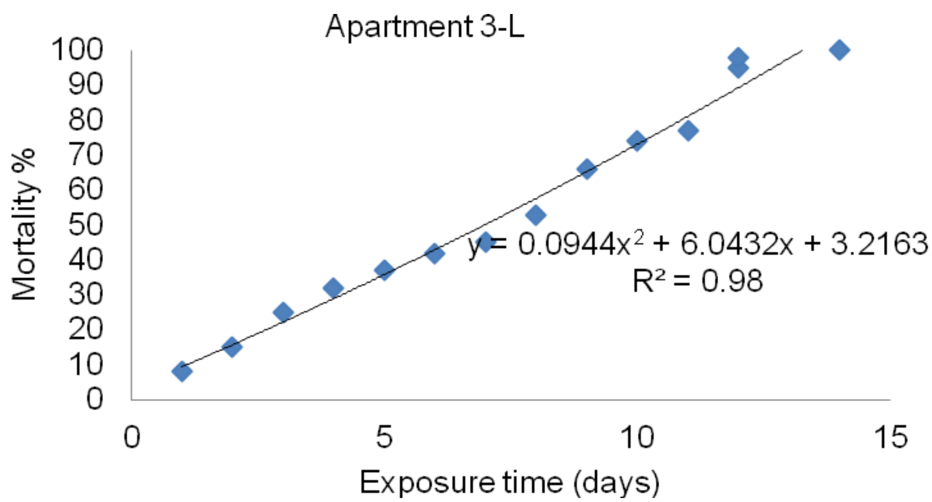

Figure 4.11 Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 3-L after 14 days of exposure.

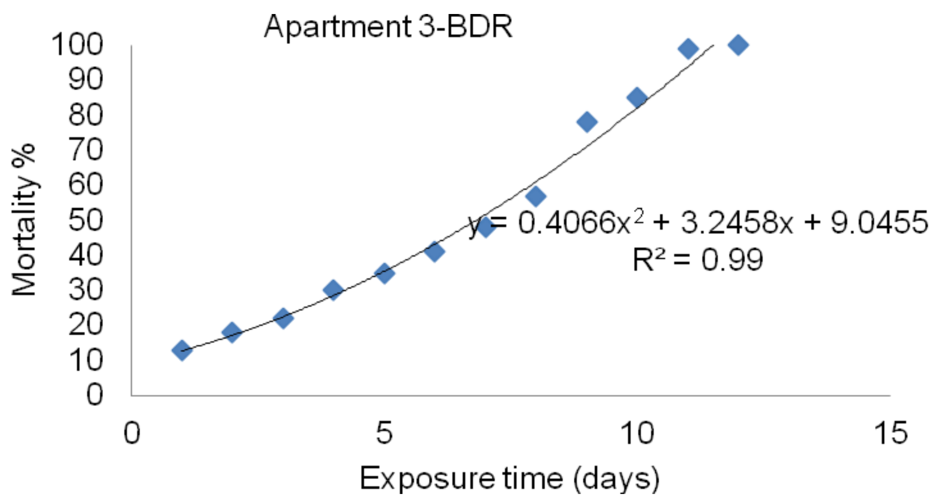

Figure 4.12. Effect of Mohlolo insecticide bait on mortality of *Blattella germanica* population in Apartment 3-BDR after 14 days of exposure.

Table 4.2 Optimum exposure days to Mohlolo insecticide bait to reach maximum mean mortality in a population of *Blattella germanica*.

Apartment room (s)	**X**	**Y**
1, K	-3.13	-7
1, L	-10,09	-30
1, BTR	-3.78	-9
1, BDR	-6.11	-16
2, K	-25.43	-62
2, L	-3.68	-17
2, BTR	-0.54	1
e3, K	-20.25	-44
3, L	-32.01	-94
3, BDR	-3.99	-5

K = kitchen, L = lounge, BTR = bathroom, BDR = bedroom

$x = -b_1/2b_2$

Table 4.3 Optimum exposure days to Mohlolo insecticide bait to reach maximum mean mortality in a population of *Blattella germanica*.

Apartment room (s)	**LT50**	**LT100**
1, K	9	14
1, L	8	14
1, BTR	8	13
1, BDR	9	14
2, K	8	13
2, L	7	12
2, BTR	10	12
3, K	10	14
3, L	7	14
3, BDR	7	12

K = kitchen, L = lounge, BTR = bathroom, BDR = bedroom

4.4 Discussion

Generally, the data collection time did not go beyond the neutral and then inhibited mortality phases. The collection of data during periods of stimulated mortality only, resulted in a situation where the values of b_1 and b_2 were each positive, resulting in a situation where $-b_1/2b_2$ remained negative. The data showed that reduction in cockroach numbers in the kitchens was higher than in bathrooms and lounge areas except in Apartment 2 and 3. The study confirms the finding by Gorham (1991) and in his study, reduction in numbers of *B. germanica* population was attributed to the biological requirements such as food, water and harbourage. Cockroach reductions were recorded from the highest trap room to the lowest as follows: kitchen > Dining > hall >living >bed room > bath room, which clearly shows that kitchens should have more traps than the other rooms (Wright and Hillmann, 1973).

Cornwell (1968) found cracks and crevices in furniture, kitchen equipment, wall voids and elsewhere in structures as primary harbourage sites for the German cockroach. These harborages often become heavily littered with cockroach feces (Stejskal, 1997). Based on the data by Rust, (1986) and Bennett *et al*., (1988) and results of previous reports, fecal deposits found in these areas may significantly reduce the efficacy of some insecticides. According to Scott (1990) and Mallet (1989), feces of cockroaches may contain microbes that could have the capacity to metabolise the insecticide. Mortality may also decrease because insects may also develop behavioural resistance by avoiding insecticides (Scott, 1990; Mallet, 1989). Behavioral resistance results from actions evolved in response to selective pressure exerted by the toxicant (Lockwood *et al*., 1984). These actions enhance the ability of a population of insects to avoid the lethal effects of that toxicant. In addition, a large number of habourages also give cockroaches more protection from pesticide and consequently apartment with lots of clutter are difficult to treat (Gupta *et al*., 1973). However, treatment of these areas with insecticides bait is a recommended method for cockroach control in food-handling establishments (Rust, 1986; Bennett *et al*., 1988).

Decreased insecticide efficacy in the presence of German cockroach feces was estimated based on results of previous reports. For example, organic matter in soil reduces insecticide toxicity by acting as an adsorbent (Hamaker and Thompson, 1972). Similarly, activated carbon has been used to protect grass seed from herbicides (Lee, 1973). Generally, the German cockroach control failures in kitchens also had been associated with insecticide affinity to cooking oils. In addition to reducing insecticide efficacy by adsorption, microbial degradation also may affect insecticide efficacy (Ree, 1980; Schal, 1988; Rust and Reierson, 1988).

The practical use of MIB as novel bait requires additional study to determine whether it can cause secondary kill. Secondary kill is the mortality of unexposed cockroaches, which can occur through contact with or feeding on poisoned cockroaches (Cannibalism, necrophagy), excretions produced from poisoned cockroaches (coprophagy), or oral secretions passed from poisoned cockroaches (emetaphagy) (Gahlhoff *et al*., 1999; Durier and Rivault, 2000; Kopanic *et al*., 2001).

4.5 Conclusions

In conclusion, MIB has the potential to control *B. germanica* population to 100% mortality due to its bait matrix palatability, toxicity and non-repellency of the active ingredient used in the bait. The bait can be used in agro processing areas or in post-harvest storage facilities to control German cockroaches.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF THE STUDY, RECOMMANDATIONS AND CONCLUSIONS

5.1 Summary

The use of baiting in cockroach management is currently the sought approach in the world where cockroaches are problematic. Bait has the advantage over liquid or dust formulations because baiting requires shorter service, has shown increased efficacy, and has reduced environmental contamination.

Chapter 3 summarises the results with the age of cockroach significantly depended on the period of exposure to the Mohlolo insecticide bait ($P < 0.05$). The period of exposure to Mohlolo insecticide bait attributed 97%, 99%, 100%, 100%, 100% and 98 of total variation in 1-, 2-, 3-, 4-, 5-d old nymphs and adult *B*. *germanica* respectively. The study showed that 1-d old *B. germanica* nymphs are more resistance to Mohlolo insecticide bait compared to 2-, 3-, 4-, 5-d old nymphs and adult *B. germanica* nymphs. According to the data, 100% mortality of 1-d old nymphs was obtained after seven days exposure to Mohlolo insecticide bait, whereas the subsequent ages could not live beyond 4 days.

The data in Chapter 4 demonstrated that reduction in cockroach numbers in kitchens was higher than in bathrooms and lounge areas except in Apartment 2 and Apartment 3. The study confirms the finding by Gorham (1991) and in his study, reduction in numbers of *B. germanica* population was attributed to the biological requirements such as food, water and harborage. Cockroach reductions were recorded from the highest trap room to the lowest as follows: Kitchen > Dining > hall >living >bed room > bath room, which clearly shows that the kitchens are likely to have greater traps than other rooms (Wright and Hillmann, 1973).

5.2 Significance of the study

This study on the efficacy of Mohlolo insecticide bait on cockroaches could be an additional contribution in the field of biology because it will offer an alternative control for costly prepared insecticide and make use of bait that is prepared by locals in the Province. This bait would also help people living in remote areas that have no financial access to purchase commercial insecticide in drug stores. The procedure or processes that would be discovered in this study would be encouraged of a new product from Mohlolo insecticide bait that is easy to administer and cost effective, and when properly prescribed and used have advantage of being relatively free of side effects.

5.3 Recommandations

The practical use of MIB as novel bait requires additional study to determine whether it can cause secondary kill. Secondary kill is the mortality of unexposed cockoraches, which can occur through contact with or feeding on poisoned cockroaches (Cannibalism, necrophagy), excretions produced from poisoned cockroaches (coprophagy), or oral secretions passed from poisoned cockroaches (emetaphagy) (Gahlhoff *et al*., 1999; Durier and Rivault, 2000, Kopanic *et al*., 2001). A chemical analysis of MIB must be conducted in order to determine the active ingredients which cause death to the experimental species of *B. germanica*.

5.4 Conclusions

Based on the results of the study, the following conclusions were drawn. MIB had insecticidal effects which cause death on both *B. germanica* nymphs and adults. This finding is very in-courageous knowing that our experimental bait which was developed using our own recipe containing bread, water and other unrevealed additives worked extremely well and apparently its efficacy in controlling *B. germanica* nymphs and adults has comparable effect to the commercial insecticides. There was significant difference between mortality of 1-day and 2-, 3-, 4-, 5- day old nymphs and adult *B*. *germanica*. However, 1-day old *B. germanica* nymphs were more resistance than the other age groups (2-, 3-, 4-, 5- day *B. germanica* old nymphs and adult).

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APPENDICES

Appendix 3.1 Analalyses of variance for different ages of cockroaches in 1-day old *B. germanica*

Source of variation	DF	SS	Percent	F-value	P-value
Days	7	22858.8	47.4	9.28	0.0000
Error	72	25330.0	52.6		
Total	79	48188.8	100		

Appendix 3.2 Analyses of variance for different ages of cockroaches in 2-day old *B. germanica*

Source of variation	DF	SS	Percent	F-value	P-value
Days	3	34460.0	72.9	32.4	0.0000
Error	36	12780.0	27.1		
Total	39	47240.0	100		

Appendix 3.3 Analyses of variance for different ages of cockroaches in 3-day old *B. germanica*

Source of variation	DF	SS	Percent	F-value	P-value
Days	1	1125.00	25.7	6.23	0.0225
Error	18	3250.00	74.3		
Total	19	4375.00	100		

Appendix 3.4 Analyses of variance for different ages of cockroaches in 4-day old *B. germanica*

Source of variation	DF	SS	Percent	F-value	P-value
Days	2	4160.0	22.0	3.82	0.0347
Error	27	14720.0	78.0		
Total	29	18880.0	100		

Appendix 3.5 Analyses of variance for different ages of cockroaches in 5-day old *B. germanica*

Source of variation	DF	SS	Percent	F-value	P-value
Days	2	3306.67	33.8	6.89	0.0038
Error	27	6480.00	66.2		
Total	29	9786.67	100		

Appendix 4.1 Analyses of variance in apartment 1 kitchen for *B*. *germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	13320.0	13320.0	376.93	0.0000
Residual	11	388.7	35.3		
Total	12	13708.8			

Appendix 4.2 Analyses of variance in apartment 1 lounge for *B*. *germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	15726.5	15726.5	702.22	0.0000
Residual	12	268.7	22.4		
Total	13	15995.2			

Appendix 4.3 Analyses of variance in apartment 1 bathroom for *B*. *germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	13081.6	13081.6	434.09	0.0000
Residual	11	331.5	30.1		
Total	12	13413.1			

Appendix 4.4 Analyses of variance in apartment 1 bedroom for *B*. *germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	15527.6	15527.6	500.97	0.0000
Residual	12	371.9	31.0		
Total	13	15899.5			

Appendix 4.5 Analyses of variance in apartment 2 kitchen for *B*. *germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	9501.24	9501.24	708.41	0.0000
Residual	11	147.53	13.14		
Total	12	9648.77			

Appendix 4.6 Analyses of variance in apartment 2 lounge for *B*. *germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	7746.55	7746.55	1442.48	0.0000
Residual	10	53.70	5.37		
Total	11	7800.25			

Appendix 4.7 Analyses of variance in apartment 2 bathroom for *B. germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	11350.2	11350.2	208.71	0.0000
Residual	10	543.8	54.4		
Total	11	11894.0			

Appendix 4.8 Analyses of variance in apartment 3 kitchen for *B. germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	11194.7	11194.7	376.27	0.0000
Residual	12	357.0	29.8		
Total	13	11551.7			

Appendix 4.9 Analyses of variance in apartment 3 lounge for *B. germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	12014.9	12014.9	555.66	0.0000
Residual	12	259.5	21.6		
Total	13	12274.4			

Appendix 4.10 Analyses of variance in apartment 3 bedroom for *B. germanica* population

Source of variation	DF	SS	MS	F	P
Regression	1	10408.4	10408.4	272.99	0.0000
Residual	10	381.3	38.1		
Total	11	10789.7			