KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

THE EFFECTS OF INSECTICIDES ON HONEY BEE (*APIS MELLIFERA*) POPULATION IN TWO SELECTED FARMING COMMUNITIES (DAMONGO AND LARABANGA) OF WEST GONJA DISTRICT IN THE NORTHERN REGION OF GHANA

BY

JOSEPH LAMBON

(PG6090611)

OCTOBER, 2015.

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IN ENVIRONMENTAL SCIENCE

BY

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OCTOBER, 2015.

DECLARATION

I hereby declare that this submission is my own work towards the Masters of Science in Environmental Science and that, to the best of my knowledge, it contains no material(s) previously published by another person(s) which have been accepted for the award of any other degree of the University or elsewhere except where the acknowledgement has been made in the text.

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HEAD OF DEPARTMENT **Signature** **Date**

DEDICATION

A SPECIAL DEDICATION

TO

AWATEY SAMUEL

WITHOUT HIS GUIDANCE THE ACCOMPLISHMENT OF THIS THESIS WOULD

NOT HAVE BEEN POSSIBLE

AND ALSO

FOR

MY WIFE: MRS LAMBON FEDELIA AND OUR SONS LAMBON AARON YUMAN AND LAMBON STEPHEN PAKINYENNU

FOR THEIR INSPIRATION, CONSTANT MORAL SUPPORT,

ENCOURAGEMENT AND LOVE

THROUGHOUT MY LIFE

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ABSTRACT

Honey bee (*Apis mellifera*) is the prominent and most economically important group of pollinators, whose populations have declined over recent years, raising widespread concern. One conspicuous threat to honey bees is their unintended exposure to insecticides. Insecticides, once absorbed into the plant; can be present in pollen and nectar, making these flora resources toxic to pollinators that feed on them. This study therefore examined the toxic effects of insecticides on honey bees in some selected farming communities (Damongo and Larabanga) in the Northern Region of Ghana. To achieve this objective, an oral interview was conducted on forty farmers from the two communities where the bees were obtained and reared in the Damongo Agricultural Training College where the study took place. The data collected showed that three types of insecticides namely Controller Super 2.5 EC, Pyrinex 48 EC, and Golan SL were the commonly used insecticides in the area. The number of dead bees recorded after application of each concentration of the various insecticides were counted and used for the estimation of LC_{50}. The results showed that Pyrinex 48 EC was the most toxic to honey bees in laboratory studies with calculated LC_{50} (1hour) value of 1.10 ± 0.37 ml/L, 1.86 ± 0.53 ml/L for Controller Super 2.5 EC, and 2.45 ± 0.83 ml/L for Golan SL. However, the mortalities of the honey bees at the various concentrations were directly related to the duration of exposure. A lower concentration of the insecticides when exposed to the honey bees for a longer period caused higher mortality than exposure of the honey bees to a higher concentration for a shorter period. Although mitigation efforts have had a limited impact, it is expected that the policy recommendations of this study if adopted and strictly adhere to will help reduce, if not completely ameliorate the unintended impact of insecticides on pollinating insects.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Honey bees (*Apis mellifera*) and their products are very useful to human survival and ecology. These uses are broad ranged and include the provision of food, their use in the area of medicine, agriculture and in folklores. The wide ranged importance of honey bees is embodied in the various hive products such as honey, pollen, propolis, bee wax, royal jelly and bees‟ venom. Honey bees produce sweet food called honey from nectar of flowers which is normally collected and eaten by humans as food (National Honey Board, 2014).

Honey has a number of constituents such as carbohydrates, dietary fiber, fat, protein, all the vitamin B types, vitamin C, water and the various essential mineral salts for healthy human growth (USDA, 2014). Honey can be eaten raw or used in many food beverages, sweeteners and flavouring (Bryant, 2001). The main uses of honey in food preparation is in cooking, baking, as a spread on bread, and addition to various beverages such as tea, porridge among others (White, 1992)

Honey also has a number of properties such as its acidity, enzyme activity, and antibacterial mechanism known as hydrogen peroxide (H_2O_2), methylglyoxal (MGO), bee defensin-1, the PH and the osmotic effect (Bradbaer *et al.,* 2004).

The many constituents and properties of honey results in its healing properties. Beetherapy which is commonly known as apitherapy is the use of bees and their products in the treatment of various ailments (Root and Root, 2005).

Honey medicine has been used historically through both oral and topical application to treat various ailments including ulcers, skin infections, gastric disturbances, wounds and burns (Pacanac, 2013). Honey is known to be effective in healing of mild burns and wounds when used in dressing them (Wijesinghe *et al*., 2009). When used in combination with many other substances, honey is found to be effective in killing cancer cells in humans (Kochan, 2014). Furthermore, honey is used as a soothing agent in the treatment of coughs and sore throat (Mulhollan and Chang, 2009). Honey also serves as a source of energy, fights exhaustion and depression and builds resistance against cold and flu (Bradbear *et al.,* 2004).

The importance of honey bees extends beyond their products to the bee itself. In some cultures, honey bees and bee larvae is eaten with rice after being mixed with shredded coconut and steamed (FAO, 2014). Bees are used in folklores to tell about important events in the household (Steve, 2006). In economic and commercial activities, bees‟ products such as honey and bees‟ wax can be sold to generate income for many households, institutions and companies. Honey bees are also widely used in advertisements especially in products containing honey.

Bees have been used as a model for the human society by some political theorists. The reputation of a community of honey bees is highly regarded from the ancient to the modern times where the bee represents hard work in the human history (Wilson, 2004).

Another very important bee product is beeswax. Beeswax has numerous uses, among which are its uses in the pharmaceutical and cosmetic industry to produce various medicines and jewelery. It is an important component of various ointments, skin creams, pills and as a carrier for other ingredients. It is also used in the manufacture of

candles, crayons, and polish for cars, furniture and shoes. In addition, beeswax can also be used to manufacture lubricants for industrial use and in the manufacture of compact disc (Woo, 2004).

According to Bradbear *et al. (*2004) propolis is another crucial product from honey bees made from gum and resins of plants. It has anti-fungal, anti-inflammation and ant-bacterial properties. It is therefore used in the treatment of cold, sores, genital herpes and post-surgery mouth pain. Propolis is used in toothpaste and chewing gums as a remedy for toothache.

Pollen obtained from plants by honey bees contain about 30% protein and a full complement of mineral salts, vitamins, trace elements, carbohydrates and fatty acids (Bradbear *et al.*, 2004).

Royal jelly is not only used by honey bees to feed the larvae and the adult queen but is also harvested and consumed by humans (Maleszka, 2008). It is a concentrated source of many nutrients such as vitamin A, C, D, E and all the B vitamins including some important fatty acids (Bradbear *et al.*, 2004).

Finally, bee venom is another important bee product which is a complex mixture of proteins, amino acids, enzymes, sugars, lipids, meltittin and one polypeptide. It has a strong antibacterial activity and therefore used in the treatment of rheumatoids arthritis and in easing inflammation ailments (Molan, 1992). It is also used to treat pain from tendon injuries, repetitive strain injuries and other muscle injuries,

(Resiman, 1994)

Pollination, which is the only means to ensure the perpetuation of the flowering plant species and to prevent extinction can only be successfully achieved with the effort of the honey bee (Jordan, 2011). Approximately 90% of all flowering plants require

pollinators to survive. According to Boland (2010), 80% of all flowering plants are entomophilies (depends on insects to be pollinated for reproduction) and half of the tropical pollinators are honey bees.

In agriculture, nearly a third of pollination is accomplished by honey bees. Honey bees find their food which is nectar and pollen in flowers and in an attempt to extract this food, they end up by pollinating the flowers. The efficiency of the honey bees" pollination is due to their large numbers, physique and their behaviour of foraging known as fidelity feeding which means they concentrate on one specific species of flowers at one time when gathering and transporting pollen and nectar. They use the dance language to communicate about a food source to their brood (Riley *et al.*, 2005). Some flowers need several visits by bees to ensure complete pollination. If fertilization is inadequate due to lack of bees, it can lead to undeveloped seeds, small and poorly shaped seeds and fruits.

Many plants reproduce vegetatively but there is a sustainability problem with this due to changes in the environment such as climatic changes and new pests and diseases emergence. There is therefore the need for genetically different plants to adapt to different changes in the environment due to special genetic constitutions by different plants. Cross pollination is therefore the only way to constantly mix the genes where bees transport pollen from one plant to the other so that the offspring become genetically different (Jordan, 2011). This provides a greater chance for some of the offsprings to survive in the competition of life. In this the honey bee is found to be one of the most important factors.

Without bees there would be no or few flowering plants and biodiversity would not be great. Bees provide better pollination that leads to improved generation of trees and conservation of the forests‟ biodiversity. In many plants as diverse as nuts, fruits and vegetables, pollination is the only chance of increasing yield whiles post-pollination inputs such as growth regulators, pesticides, fertilizer and water are actually designed to preserve quality and prevent losses (Hackett, 2004). Bees pollinate more than 16% of the flowering plant species to ensure that we have blooms in our forests and gardens (Hackett, 2004).

Pollination is so important that man cannot afford to risk losing honey bees which are the most dominant pollinators in nature. Losing them will threaten our diet staples from flowering plants, and even our beef and dairy industry which also depends on these plants (Boland, 2010). According to Al-Hassan and Diao (2006), the estimated yield gap for most traditional crops in Ghana ranges from 200% to 300%. For farmers to increase productivity in order to meet this yield gap, they use pesticides which tend to affect bee population. The increased use of pesticides has drastically reduced the honey bee population. Many bee poisoning problems could be prevented by better communication, education and cooperation among the growers, pesticide applicators and beekeepers (Grubb, 2013).

A number of sources have reported declines in certain pollinators‟ species globally. Every continent, except for Antarctica, has reports on pollinator declines in at least one region or country (Alan, 2013). The losses of pollination services have been well documented in many specific instances. According to Alan (2013) the United State Department of Agriculture, National Agriculture Statistics Survey, managed honey bee colonies have declined from a peak of approximately 6 million colonies in 1947 to roughly 2.5 million in 2006. The report noted that insufficient information existed to

determine the causes of those declines. Similar declines were also noted in Europe (Biesmeijer *et al.,* 2006). According to Williams and Osborne (2009) more than half of important managed pollinators, honey bees *(Apis mellifera)* colonies in the UK are rare or in decline.

According to Pettis *et al.* (2012) the pollinator declines are as a result of multiple factors which may be acting in various combinations. Rortais *et al. (*2005) had hypothesized that some of these factors may interact and result in loss of bees. Although the exact causes of this decline are still currently analyzed, it is admitted that the extensive use of pesticides against insect pests for crop protection has contributed to the loss of many pollinators. Adult bees may be exposed directly to insecticides through direct overspray or flying through spray drift, by consumption of pollen and nectar (which may contain directly over-sprayed or systemic residues), by contact with treated surfaces (such as resting on recently treated leaves or flowers), by contact with dusts generated during drilling of treated seeds, or by exposure to guttation fluid potentially as a source of water or as dried residues on the surface of leaves (Krupke *et al.,* 2012)**.**

Although insecticides have not been implicated as the singular cause of insect pollinator decline in general or decline in honey bees‟ population specifically; efforts have been directed at determining the extent to which pesticides may be affecting bees and ways to mitigate potential effects in the advanced countries. Regulatory authorities in North America and elsewhere are developing improved procedures for evaluating the potential risks of pesticides to bees. Survey of managed migratory bees indicates that a broad range of insecticides have been detected in hive products (e.g. honey, stored pollen, wax). Typically, combinations of insecticides are detected in the same hive products, with an average of four insecticides detected in the same sample (Mullin *et al.,* 2010).

There has not been sufficient study to quantify the effects of pollinator decline in Ghana irrespective of international initiatives such as the International Pollinator Initiative (IPI) which highlight the need for public awareness and participation on pollinator protection by encouraging the practice of bees‟ conservation. Some plants on the endangered species list are endangered because they have lost their normal native pollinator.

Bees have the potential to become keystone indicator species of environmental degradation. Any changes in their abundance and diversity will influence the abundance and diversity of the prevailing plant species. There is a mutual dependency as bees rely on a steady nectar source and pollen source throughout the year to build up their hive (Morse and Calderone, 2000).

Ecosystem services are put at risk as a result of indiscriminate use of external inputs such as insecticides, and indeed it is well-recognized that beneficial insects such as pollinators may be negatively affected by these insecticides. Risk assessment procedures for honey bees have been well elaborated as part of pesticide evaluations based on the guidelines of the European and Mediterranean Plant Protection Organization (EPPO, 1993).

However, the registration procedures for insecticides are based on information related to only one pollinator species, the European honey bee, and are not generally fieldtested in most developing countries such as Ghana before the insecticides are registered. As a result, insecticides whose toxicity against local pollinators has never been tested are in widespread use.

1.2 Problem Statement

Bees, including honey bees *(Apis mellifera)* are the prominent and economically most important insect worldwide; producing honey, pollen, royal jelly, propolis and wax. In addition, thirty five (35) percent of the world food crop production depends on pollinators (Klein *et al., 2007*, Velthuis and van-Doorn, 2006).This accounts for an annual value of 153 billion Euros (Gallai *et al.,* 2009). Bees again serve humanity indirectly by contributing to the healthy functioning of unmanaged terrestrial ecosystems. However, the populations of honey bees throughout the world have been declining for more than a decade (Goulson *et al.,* 2008, van Engelsdorp *et al.,* 2010) raising widespread concern (Potts *et al.,* 2010).

The decline of these pollinating species can lead to a parallel decrease of plant species or vice versa (Biesmeijer *et al.,* 2006; National Research Council of the National Academies, 2007; Goulson *et al.,* 2008). More specifically, there is a great deal of concern about the decline of the honey bee across the world that has been termed "Colony Collapse Disorder (CDD)" (Oldroyd, 2007; VanEngelsdorp *et al.,* 2010). The abundance of pollinators in the environment is influenced by biotic factors (predators, pathogens, parasites, competitors, availability of food resources) and abiotic factors (climate, pollutants).

The detrimental factors affecting bee populations are likely to be multiple and interacting (Williams and Osborne, 2009) but one conspicuous threat is their unintended exposure to agricultural insecticides that protect crops from insects and diseases (Desneux *et al.,* 2007). The routes of exposure of bees to insecticides have been assessed (US EPA, 1995). Colonies of honey bees often are exposed to insecticides

when foragers gather contaminated nectar and pollen in the field, and return with it to the hive where it is stored and shared among nest-mates.

Northern Ghana has been described as the food basket of the nation and contributes up to 80% of Ghana‟s food basket (ACDEP, 2010). This is due to the large scale agricultural activities that go on there. In a bid to increase crop yields, farmers tend to use insecticides. A brief interaction with farmers in two farming communities (Damongo and Larabanga) in the West Gonja District where insecticides are widely used revealed that in recent times, they record heavy losses in crop yield from their farms even though they ensure that all necessary inputs are always administered.

Crop losses due to pests are clearly the greatest major impediment to sustaining production. Insecticides use has increased over time in Ghana and is particularly elevated in the production of high-value cash crops and vegetables (Kwapong, 2006). Insecticides are often taken as the first line of defense against pests, yet they also impact on at least two of the key ecosystem services that sustain crop yields: natural enemies of pests and pollinators.

James and Xu (2012) noted that insecticides can affect immunity and make colonies more vulnerable to loss from disease-causing agents. According to Alaux *et al.* (2010) and Pettis *et al.* (2012), there is positive association between spore numbers of the intracellular microsporidian parasite (*Nosema apis and N. ceranae*) in worker bees and pesticide exposure. Wild and managed bees are important pollinators whose populations have declined over the recent years (Johansen and Mayer, 199). According to Hackett (2004), 35% of all food crops directly or indirectly depend on pollination by honey bees. Further, the value of honey bee pollination to agriculture is more than $14 billion annually. Crops ranging from nuts to vegetables and as diverse as apple, cereals,

cranberry, pumpkin, and sunflower all require pollination by honey bees. Quantifying population-level response of the various exposure routes of honey bees to pesticides provides an important basis for assessing its potential for ecological impact (Wu, *et al.*, 2011).

1.3 Justification of the Study

In recent years, numerous studies have been performed to assess whether insecticides could be harmful to honey bees. In spite of the evidence of certain pesticides proven to be capable of causing population declines in non-target species (VanEngelsdorp *et al.*, 2010) studies found no correlation between the incidences of pollinator declines with the use of any insecticides. However, Creswell (2011) noted that these trials only had sufficient statistical power to detect severely detrimental impacts; so it remains uncertain whether environmentally realistic exposures are capable of making demographic impact on bees‟ populations.

In protecting the sustainability of non-target species, this study was particularly interested in establishing whether a realistic level of exposure to insecticides is capable of causing the population of honey bees to decline. This research therefore sought to determine whether some commonly used agro insecticides in Northern

Ghana can cause lethal effects on honey bees.

1.4 Main Objective

The focus of this study was to determine the effects of insecticides on honey bee population in two selected farming communities (Damongo and Larabanga) in the Northern Region of Ghana.

1.4.1 Specific Objectives

The specific objectives of the study were to:

i. determine the types of insecticides commonly used in the two communities through interviews.

ii. collect honey bees from the two communities and expose the honey bees to three of the commonly used insecticides (Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL) and determine the mortality of honey bees due to exposure to the insecticides at different concentrations.

iii. determine the acute toxicity of the insecticides when applied to honey bees.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Honey Bee Life Cycle

The reproductive cycle of a worker honey bee is approximately 21 days. Although the cycles are slightly different for drones (24 days) and queens (15 days), the vast majority of bees in any colony are female workers, so they are typically used as the standard.

The cycle begins when a fertile queen lays an egg in a wax cell that has been prepared by the house bees. After about three days the shell and the larva emerges to float in a pool of royal jelly that has been secreted into the cell by the nurse bees. This royal jelly is produced by several glands in combination, but 60 to 80% of the royal jelly is secreted by the hypo pharyngeal gland and about 20 to 40% is secreted by the mandibular gland (Sammataro and Avitabile 1998).

During a worker bees‟ time as a nurse; this worker bee eats and digests large quantities of pollen and nectar. The body of the worker bee then, produces the glandular secretions

that nourish the larvae. According to Oliver (2010), the larvae grow quickly during the next 6 days increasing their body weight 1500 to 1700 times. As the larvae mature, the nurse bees gradually withhold the part of the diet secreted by the mandibular glands and increase the amount of bee bread and nectar (Sammataro and Avitabile, 1998). Towards the end of the 6th day, the worker larvae even receive some whole pollen grains. Larvae defecate only once, right at the end of the larval stage. At this point each larva spins a cocoon within their wax cell, and the worker bees cover each cell with a wax coating. This is the beginning of the cocoon stage (Kevan *et al.*, 2007).

The cocoon stage lasts 12 days. While in their cocoons, the pupae undergo complete metamorphosis, changing from the larval stage to adult bee. They do not eat during this period, but use the food they stored as larvae to form their new bodies. Soon after the young workers emerge, they go to work, cleaning the cells in which they were born.

As the adult worker matures, it performs a number of other tasks. These tasks are performed sequentially, changes with aging of the adult worker bee, although its task may also change with the colony‟s need. For instance, the adult worker bee might spend the first day or two as a house bee, cleaning and polishing the brood cells, then spend the next 7 or 8 days as a nurse; feeding and caring for the young. After that the adult worker bee may tend the queen and build comb (Batra, 1995). Later the adult worker bee may become a guard; monitoring bees as they come and go to assure that foreign bees are not admitted. Guard bees also attack intruders. The last stage of a worker‟s life is that of a forager. A forager may collect water, pollen, nectar, or propolis, depending on the colony‟s need. Foragers work until they wear themselves out, devoid of energy and tattered of wing, they usually die in the field.

2.2 The Colony Reproductive Cycle

While individual bees are born and die on a relatively short cycle, the colony as a whole operates on a much different calendar. In spring, when a queen begins laying eggs in earnest, the hive population increases rapidly along with the availability of nectar and pollen. The queen can lay about 2000 eggs per day, and the colony can increase from a few thousand to tens of thousands of bees in several weeks (Tautz, 2008). According to Sammataro and Avitabile (1998) a summer colony may contain 40,000 to 70,000 members, of which 40% to 45% are brood and 55% to 60% are adult workers. The rest of the bees are male drones. Drones have no duties except to mate with young queens from other hives. They perform no hive chores, nor do they collect provisions or build comb. They are devoid of stingers and therefore cannot defend the hive.

At the peak of spring build-up, the colony may split into two parts, thus beginning a new colony. This phenomenon, called swarming, occurs when over-crowded worker bees produce a second queen. When this queen is nearly ready to lay, the old queen along with about 40% to 70% of the workforce leave the hive to take up residence in a new location, and the new queen reigns over the old hive (Tautz, 2008). Thus, in honey bees, whole-colony reproduction occurs as well as individual bee reproduction. When food is abundant in the environment, egg-laying is greatly reduced, but collection and storage of supplies continues unabated as long as the weather permits. During the dry season when there is less food in the environment, the colony may be down to about 10,000 members. These bees are the ones who will tend the queen and sustain the colony during unfavourable conditions. Bees do not hibernate but actively work to keep the colony warm and the queen healthy (Sammataro and Avitabile, 1998).

2.3 The Spate of Insecticides Usage in the World

Insecticides are used in agriculture, medicine, industry and the household. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century (Cooper and Hans, 2007). Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; and others are concentrated in the food chain (Plimmer and Johnson, 1991).

There are several ways through which honey bees can be exposed to insecticides in the environment. Wind and runoff from rain may move insecticides from their original place of application to other areas where they may contaminate the atmosphere, surface water, ground water and the soil (Plimmer and Johnson, 1991). Atmospheric and oceanic currents may also carry insecticides to long distances away from where they were manufactured or used which can be accumulated in food chains (Kanga, 1980).

In crop production, insecticides are used to control insects and diseases. They are usually applied directly to the crops and some could remain as residues on or in leaves and flowers, or fruits and seeds after they have been harvested. Honey bees which therefore depend on these plants for nectar and pollen are exposed to these insecticides through the various routes such as contact, oral etcetera (Odoux *et al*., 2012).

Several plant protection products are dangerous to honey bees and other pollinators in many ways (Riedl *et al.,* 2006; Desneux *et al.,* 2007). Therefore both active substances and formulated pesticides currently undergo various tests to assess the risk posed by them to honey bees, before their use in agriculture is allowed. For doing so, the European and Mediterranean Plant Protection Organization guidelines No. 170

(OEPP/EPPO, 2010a) and the relative risk assessment scheme (OEPP/EPPO, 2010b) are usually followed in the European Union. Such procedures substantially rely on Median Lethal Dose (LD_{50}) or other similar toxicity index determination in order to ascertain if risk levels associated with the tested active substance are acceptable for honey bees.

About 759 chemical and biological pesticides are used worldwide in agricultural and health sectors. Data compiled by the World Health Organization (WHO, 1998) in the United States of America (USA) had classified 33 pesticides of the 759 chemical and biological pesticides as extremely hazardous to human health and the environment (class Ia), 48 as highly hazardous (class Ib), 188 as moderately hazardous (class II), and 239 as slightly hazardous (class III). One hundred and forty-nine (149) pesticides have been considered as unlikely to cause acute hazard in normal use (class IV). Honey bees are thought to possibly be affected by such chemicals which are known to work their way through the plant up into the flowers and leave residues in the nectar and pollen which bees forage on (Hackett 2004).

Previous studies have shown a relationship between insecticide residues in food products and health problems such as cancer, weakened immune system, nervous system disorder, and attention-deficit (hyperactivity) disorder. Some insecticides also contain endocrine disrupting chemicals (EDs) which have a significant effect on the body‟s hormonal system and can mimic the body‟s natural hormones thereby causing adverse health effects through disruption of normal body functions (Oldroyd, 2007). Recent studies have linked insecticides exposure to bee health decline (Kunin, 2006). Odoux *et al.* (2012) used Radio-frequency identification (RFID) to test the hypothesis that a sublethal exposure to pesticide cited in the legal petition; neonicotinoid indirectly

increases hive death rate through homing failure in foraging honey bees. When exposed to sublethal doses of thiamethoxam, at levels present in the environment, honey bees were less likely to return to the hive after foraging than control bees that were tracked with RFID which were not intentionally dosed with pesticides. The survival rate was even lower when exposed bees were placed in foraging areas with which they were less familiar.

Penelope *et al.* (2012) also exposed colonies of the bumble bees to levels of imidacloprid that were realistic in the natural environment, and then allowed them to develop naturally under field conditions. Treated colonies had a significantly reduced growth rate and suffered 85% reduction in production of new queens compared to unexposed control colonies. The study was particularly noteworthy because it showed that bumble bees, which are wild pollinators, were suffering similar impacts of pesticide exposure to "managed" honey bees.

As a result of these globally recognized concerns of pollination decline, the Food and Agriculture Organization of the United Nations (FAO) had coordinated the development and implementation of a global project on pollination services. The project was developed in collaboration with seven developing countries: Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa. One of the priorities of this project – as identified by the participating countries – has been to develop a protocol to identify and assess pollination deficits from a farmer"s perspective (FAO, 2014). This protocol has been applied in the seven participating countries, and it was discovered that management practices to ensure abundant pollinators can increase fruit production in mango orchards in Ghana by 35%, improve the production of mustard seed in Nepal by 25 %, and increase the canola oil content in rapeseed by 8

% in Brazil (FAO, 2014).

2.4 Insecticide Usage in Ghana

Insecticides are widely used in several areas of modern agriculture because they are considered economically important for high yield. In Ghana pesticide usage in agriculture over the past ten years has risen rapidly (Hodgson, 2003). It is estimated that pesticide use in Ghana has increased in recent times and particularly highest for the cultivation of vegetables and high income earning cash crops (Gerken *et al.*, 2001).

Despite the importance of honey bees, the effect of insecticide exposure on colony health has not been systematically monitored, and the Environmental Protection Agency (EPA) of Ghana does not require data on sublethal effects for insecticides or class of pesticides registration in Ghana.

Pesticides used in agriculture had been noted by Palmer *et al.* (2007) as the singular causal of brain damage in honey bees. Neonicotinoids and coumaphos insecticides in a laboratory were found to be target areas for bees‟ brains damages.

Insecticides use in Ghana is mainly centered on vegetables, fruits and cocoa. Some commonly cultivated crops in Northern part of Ghana to which pesticides are used includes; tomato, cabbage, garden egg and cotton which are important crops for small-scale farmers. Gerken *et al*. (2001) had noted that these insecticides are either misused or over used on these crops which could result in numerous negative effects on non-target organisms‟ productivity, human health and the environment.

The use of organochlorines has been banned in Ghana. However, they have been detected in foods, fish, meat, water, human blood and breast milk (Afful *et al.*, 2010).

This may be due to the fact that they are still being manufactured and used illegally or they have long environmental half-lives and persist in the environment years after their application.

The use of insecticides is not limited to crop production only. Along the banks of the Tano and Pra rivers in Ghana, insecticides are being used to control black flies (Ntow *et al*., 2006). In public health, temephos is being used by the Onchoccerciasis Control Programme in the Volta Basin for the control of black flies (*Simulium spp.*) which transmit Onchocerciasis (African river blindness) to humans and also to control domestic pests such as cockroaches, flies, mosquitoes, ticks and other insects (Ntow *et al*., 2006).

2.5 Effects of Insecticides on Non-Target Species

An insecticide is any substance or mixture of substances used to destroy, suppress or alter the life cycle of insects. An insecticide can be a naturally derived or synthetically produced substance. Insecticides belong to a wider group of substances called pesticides which can be in a form of an organism, for example, the bacterium *Bacillus thuringiensis* which is used to control a number of insects, or even a genetically modified crop pest (IUPAC, 2006).

Insecticides are produced in different forms: dusts and granules for dusting, liquids for spraying, powders for mixing with liquids and spraying, coatings on seeds, pellets. Contact insecticides are usually sprayed on plants and can kill bees when they crawl over sprayed surfaces of plants or other media. Actual damage to bee populations is a function of toxicity and exposure of the compound, in combination with the mode of application. A systemic pesticide, which is incorporated into the soil or coated on seeds,

may kill soil-dwelling insects as well as bees that are exposed to the leaves, fruits, pollen, and nectar of the treated plants (Alaux *et al*., 2010).

According to Hunt *et al.* (2012), insecticide toxicity is generally measured using acute contact toxicity values LD_{50}; that is the exposure level that causes 50% of the population exposed to die. Toxicity thresholds are generally set at;

- highly toxic (acute LD50 < 2µg/bee)
- moderately toxic (acute LD50 2 - 10.99µg/bee)
- slightly toxic (acute LD50 11 - 100µg/bee)
- Nontoxic (acute LD50 > 100µg/bee) to adult bees.

The recent sequencing of the honey bee genome provides a possible explanation for the sensitivity of honey bees to insecticides; relative to other insect genomes, the honey bee genome is markedly deficient in the number of genes encoding detoxification enzymes (Desneux *et al*., 2007). This notable difference renders honey bees more susceptible to insecticides than other insects, and beekeeping has been negatively impacted by insecticides applied to crops for as long as pesticides have been used. Some insecticides have been banned due to the fact that they are persistent toxins which have adverse effects on animals and humans. An often quoted case is that of DDT. One of the better known impacts of DDT is the reduction of the thickness of egg shells of predatory birds. The shells sometimes become too thin to be viable, causing reductions in bird populations. In recent times, a number of global conservation programs have also arisen to protect all countries from environmental contaminants. An example of one such program is the Stockholm Convention on persistent organic pollutants (POPs) which is a global treaty.

Colony collapse disorder (CCD) is a recent, widespread phenomenon affecting honey bee colonies in the Northern hemisphere. It is characterized by a sudden disappearance of honey bees from the hive. The syndrome is mysterious in that there are often no corpses found, and although there are often many disease organisms present, no outward signs of disease, pests, or parasites exist (Oldroyd, 2007). Multiple causes of CCD have been proposed, such as combinations of pesticides, pathogens, parasites and natural habitat degradation.

2.6 Importance of Bees

The role of bees in sustaining ecological balance of other organisms cannot be underemphasized. Three protection goals are identified: pollination services, honey production and biodiversity.

2.6.1 The Role of Honey Bees (*Apis Mellifera)* in Pollination

Pollination services are provided both by wild free- living organisms mainly bees, commercially managed bee species and a few other animals such as butterflies, moths and flies (Velthuis *et al.*, 2006). The production value of one tone of pollinator dependent crop is approximately five times higher than one of those crop categories that do not depend on insects. The total economic value of crop pollination worldwide has been estimated at €156 billion annually (Gallai, *et al.*, 2009).

Further research findings in modern days has it that crops with greater pollinator dependence have shown lower growth in yield variability relative to less pollinatordependent crops (Kwapong , 2006).

Data compiled by the Food and Agriculture Organization of the United Nations (FAO, 2014) documented a 45% increase in the global stock of domesticated honey bees

during the last five decades. While that seems quite positive, at the same time there has been a much more rapid (more than 300%) increase in the fraction of agriculture that depends on animal pollination during the last half century. So, this means that their global capacity to provide sufficient pollination services may be stressed, and more pronouncedly in the developing world than in the developed world (Aizen and Harder, 2009).

Very importantly, bees pollinate fruit crops and their demise has a serious effect on fruit farming industry and the supply of food generally. Bee declines can result in loss of pollination services which have important negative ecological and economic impacts that could significantly affect the maintenance of wild plant diversity, wider ecosystem stability, crop production, food security and human welfare (Potts *et al*., 2010).

The importance of pollination by bees goes far beyond agriculture since bees also pollinate more than 16% of the flowering plant species ensuring blooms in gardens (Hackett, 2004). Pollinators are therefore key component of global biodiversity, providing vital ecosystem services to crops and wild plants.

2.6.2. Production of Honey by Honey Bees

Honey is a sweet food made by bees using nectar from flowers through the process of regurgitation and evaporation. They store the honey as a food source in wax honey combs inside the bee hive (Kasina, 2011). It has attractive chemical properties for baking and a distinctive flavour that leads some people to prefer it over sugar and other sweeteners.

It is also used as a sweetener in some commercial beverages. Honey is the main ingredient in the alcohol beverage called mead which is also known as “honey wine” or “honey beer” (Robert, 1986). Honey contains invert sugar that has the quality of providing instant energy when consumed. Medically, honey has been used successfully in the treatment of diabetic ulcers when the patient cannot use tropical antibiotics in the tropics.

Antioxidants in honey have even been implicated in reducing damage to colon in colitis in a study involving administering honey animas to rats. Honey appears to be effective in killing drug-resistant biofilms which are implicated in chronic rhino sinusitis (American Academy of Otolaryngology, 2008). According to Ashman (2009) research in Purdue University revealed from their findings that honey is a catalyst to calcium absorption in animals.

Crop losses to pests are clearly the greatest major impediment to sustaining production. Insecticides are often taken as the first line of defence against pests, yet they also impact on at least two of the key ecosystem services that sustain crop yields: natural pest control and pollination. It is undisputable how essential pollinators are to the world‟s ecosystems in general and horticultural crop production specifically. The services that bees and other pollinators provide freely to agriculture have been taken for granted in the past. But as agriculture has intensified, with larger fields and greater applications of agrochemicals, populations of pollinators have shown steep declines in a number of localities. Insecticides are important agricultural tools often used in combinations to avoid resistance in target pest species, but there is growing concern that their widespread use contributes to the decline of pollinator populations.

Notwithstanding the above, pesticide use reduces biodiversity, contributes to pollinator decline, destroys habitat and threatens endangered species (Palmer *et al*., 2007). It is a label violation to apply most insecticides on crops during bloom, or to allow the pesticide to drift to blooming weeds that bees are visiting. Yet such applications are frequently done, with little enforcement of the bee protection directions. Insecticides misuse has driven beekeepers out of business, but can affect native wild bees even more, because they have no human or strict regulations to protect them from these harmful chemicals.

Honeybee populations are in jeopardy in crop-growing areas especially vegetables, since they are dosed repeatedly when insecticides applicators apply insecticides on blooming crop fields while the bees are foraging(Hunt, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at Damongo Agricultural Training College in the West Gonja District of the Northern Region of Ghana (Fig. 2).The District shares boundaries with Wa East District in the North West, Central Gonja District in the south, Sawla-Tuna-Kalba Districts in the West, Tolon Kumbungu District in the East and West Mamprusi District in the North (Fig. 1).

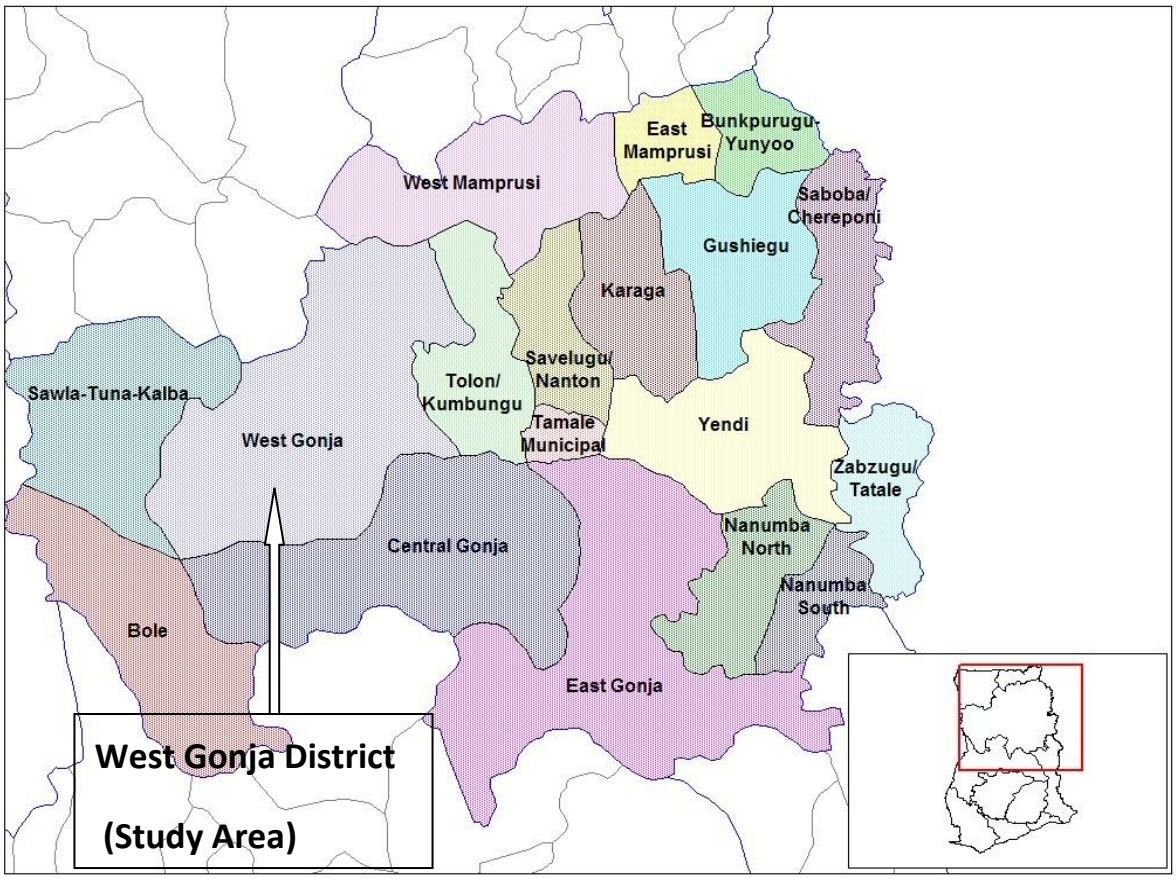

Fig 1: A map of Northern Region of Ghana showing the West Gonja District.

The West Gonja District lies between latitudes 8º32‟N and 10 º2‟N, and longitudes 1º5‟W and 2º58‟W. Damongo town lies within latitudes 9° 5' 0" N and longitudes 1° 49' 0" W whilst the Larabanga town lies latitudes 9° 13' 0" N and longitudes 1° 51' 0" W.

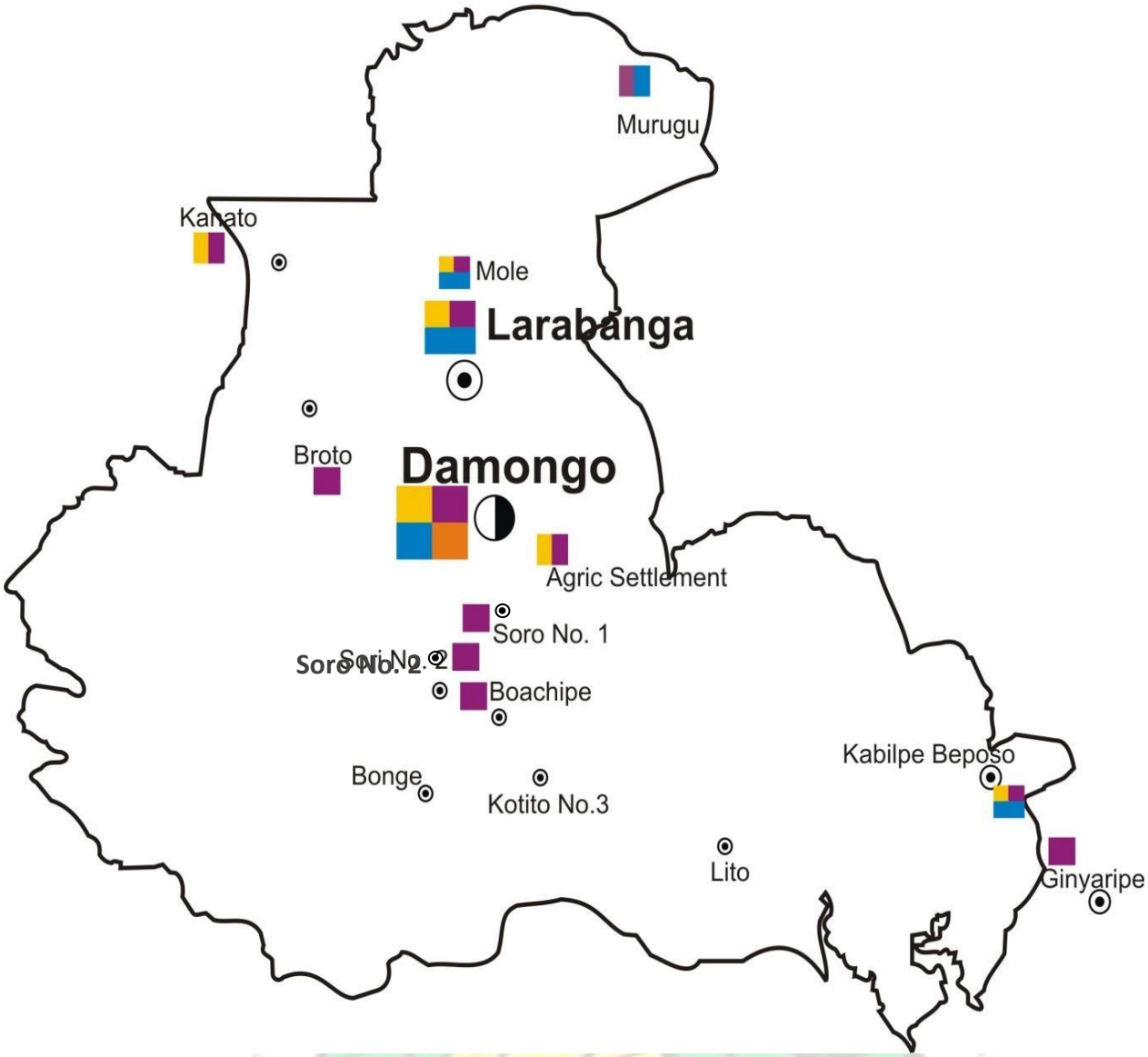

Fig 2: Map of the West Gonja District showing the study areas (Damongo and Larabanga

The district covers a land area of about 8,352 sq. km representing about 12% of the total land area of the Northern Region. The district has two forest reserves and these are the Mole National Park and Kenikeni Forest Reserve both with a rich array of flora and fauna. The Mole National Park which is located about 30 km west of Damongo, is the largest in the country and occupies a land area of 3800 km.^{2} It serves as home for various wildlife species including honeybees (Briggs and Philip, 2007). It has an altitude of

between 150 and 200 meters above sea level and a generally undulating terrain with the Damongo Escarpment as the only high land.

The area generally records high temperatures with the mean monthly temperature being 27°C. Humidity is very low with the average being 50 percent (West Gonja District Assembly, 2010).The natural vegetation is Guinea Savanna with scattered trees except in most valleys where isolated woodland or forest are found. Most trees are deciduous shedding their leaves during the dry season to conserve water (West Gonja District Assembly, 2010). The area is suitable for cultivation of crops such as millet, sorghum, maize, groundnuts, vegetables and root crops.

3.2 Sampling Site

Bees were collected from Damongo and Larabanga in the West Gonja District and reared at the Damongo Agricultural Training College. The bees were collected and used for the study in July and August, 2013.

3.4 Method

An oral interview was conducted to identify the most commonly used insecticides in the two farming communities (Damaongo and Larabanga). Forty farmers were interviewed from the two communities (i.e. twenty farmers from each community) on the subject "What type of insecticides do you often use in your farm?" Questions used for the interview can be found in appendix twelve. Using the data, the environmental realistic values of the various insecticides were determined using Microsoft Excel.

Pearson‟s correlation analysis was conducted to investigate relationships between different concentrations levels used.

3.4.1 Construction of Test Cages

Sixteen wooden cages were constructed (Plate 1) to carry out the study. Cages were constructed using 3.81 cm nails with the assistance of a professional carpenter from the Damongo Agricultural Training College. Each cage measured 11.43 cm by 11.43 cm square. The four sides of the cages were covered with 1.27 cm thick plywood and the top covered with 0.2 cm nylon mesh to provide enough ventilation for the bees and proper vision while the bottom was left opened.

Plate 1: Constructed wooden cages ready to be used for the study

3.4.2 Collection and Preparation of Bees for the Study

Live adult bees were obtained from beehives by a man wearing mask at 2:00 am (in the morning of the experiment) when the bees were outside the hive and not aggressive (Plate 2)

Plate 2: Bees resting outside their hive just before collection

The bees were collected by hand and placed into a perforated plastic container (Plate 3) and were immediately transported from the site of collection to the experimental site. The twelve wooden cages were put into four groups with each group containing three cages. The open end of each cage was placed on a flat floor and 12 to 15 bees were released from the perforated plastic container into each cage through that side by gently lifting it (Plate 4). This was done with the aid of torchlight. They were allowed to acclimatize to the experimental conditions for a period of three hours. They were maintained under standard room conditions (natural darkness) at a room temperature of 24 °C and a relative humidity of 49% throughout the study. On the morning of the experiment, the bees were reduced to ten in each cage after the dead and moribund bees were removed from each cage and where necessary, replacements were made (plate 4).

Handling procedures including preparation of concentrations, administration, observations and recording were conducted during the day. All collections and experimentations were done in August and September to coincide with the right environmental conditions for field applications.

Plate 3: Bees being collected from their hive into a perforated plastic container

3.4.3 Preparation of concentrations and administration

The method used for the calculations and preparation of the various concentrations of the insecticides and application was according to the recommended formulations on the labels on the various plastic bottles by the manufacturers of the various insecticides for field application. A clinical syringe was used to measure the calculated concentrations of each insecticide into a one litre calibrated spraying bottle containing 200 ml of water as a carrier (plate 5). The solution in the litre calibrated spraying bottle was topped up

to one litre mark. The same procedure was used to prepare all the other concentrations (Table 1).

Plate 4: Insecticide applied to bees in various wooden cages

Table 1; Concentrations of insecticides used for the study

Insecticide Type	**Concentration ml/L**				
Controller Super 2.5 EC,	1.0	1.7	3.30	5.00	6.7
Pyrinex 48 EC	0.5	1.0	1.5	2.0	2.5
Golan SL	0.5	1.0	1.5	2.0	2.5

A control solution was prepared for each dosage concentration using one litre of distilled water only.

Plate 5: Measurement of various concentrations

3.5 Application of Doses

Each formulated insecticide was gently sprayed on top of each test cage containing ten bees. This was repeated in the other two cages to cover the set of three cages for each insecticide. The spraying bottle was then immediately rinsed several times with clean water after which the process was repeated for the other two insecticide brands. One litre of distilled water was then poured into a well washed and rinsed spraying bottle and used to spray a set of three other test cages containing ten bees each to serve as control. This was repeated for the remaining concentrations on different days.

3.5.1 Test Duration and Observations

The test lasted for 90 minutes for each concentration administered with their respective controls but recordings ended at the 60th minute since it was observed that no mortalities occurred between 60 to 90 minutes for all the concentrations applied.

3.5.2 Determination of recommended formulated values

Using the data, the recommended formulated values of the various insecticides were determined using Microsoft Excel. Pearson‟s correlation analysis was conducted to investigate relationships between different concentrations levels used.

3.6 Validity of the Test

For the test to be valid the following conditions were observed:

- Only healthy adult live bees were used for the test. Bees were collected and kept under field conditions for three hours before application of the various doses.
- Preparations of all doses were done using the prescribed formulations for the application of the various insecticides to specific crops in the field.
- The average mortality for the total number of controls did not exceed 10 per cent at the end of each test session.
- The LC_{50} of the toxic standard met the specified range.
- All instruments used for the test were always washed with a detergent and hot water, rinsed with tap water and finally with distilled water before use. After using them for a particular dosage, the same was repeated before using them for the next one.

3.7 Percentage mortalities and calculation of LC_{50}

The bees were observed at ten minutes interval for ninety 90 minutes for any toxic signs. The number of dead bees in each cage was counted and the percentage of mortality was calculated using the graphical method of Tainter and Miller (1944). The percentage of bees that died at each dose was then transformed to probit using Finney‟s method (Finney, 1952). The probit values obtained were plotted against logconcentration and the concentration corresponding to probit 5, i.e., 50% was found. The graph obtained gave the probit versus log-concentration Curve. The S.E of LC_{50} was calculated using the formula of Ghosh (1984).

Approx S.E of $LC_{50} = \frac{\log LC_{84} - \log LC_{16}}{\sqrt{2N}}$

3.7.1 Estimation of Acute Toxicity of the Insecticides when Applied to Honey Bees

The standard method to evaluate the toxicity of the insecticides that could potentially be in contact with the honey bees consisted of the calculation of an acute toxicity data. The acute toxicity of an insecticide was determined by the calculation of median lethal concentration (LC_{50}), that is, the concentration that will kill 50% of animals of a particular species.

The corrected % Formula for 0% mortality and 100% were calculated using the formula of Ghosh (1984)

For 0% dead = 100 * (0.25/n)

For 100% dead = 100 - (100*0.25/n).

Where n is number of honey bees in each group

3.8 Median Lethal Concentration (LC_{50}) of Controller Super 2.5 EC

Three groups of adult bees of 10 bees in each group were placed in wooden boxes. Five different concentrations of 1.0 ml/l, 1.7 ml/l, 3.3 ml/l, 5.0 ml/l and 6.7 ml/l in the case of Controller Super 2.5 EC were applied. The numbers of bees with behavioral modifications or dead during 10, 20, 30, 40, 50 and 60 minutes were recorded.

The percentage of honey bees that had died at each concentration level was then transformed to probit (Table 12 in appendix 11). A control group experiments were performed using distilled water (Table 2 in appendix 1).

The probit values were plotted against log-concentrations (figure 3); the concentration corresponding to probit 5, that is, 50% was found.

3.9 Median Lethal Concentration (LC_{50}) of Pyrinex 48 EC

In the case of Pyrinex 48 EC, five different concentrations (0.5 ml/l, 1.0 ml/l, 1.5 ml/l, 2.0 ml/l and 2.5 ml/l) were applied to the three groups of adult bees (10 bees in each group). The number of bees with behavioral modifications or dead during 10, 20, 30, 40, 50 and 60 minutes was recorded. The percentage of bees that had died at each concentration level was then transformed to probits (Table 12 in appendix 11). The probit values were plotted against log-concentrations (figure 4); the concentration corresponding to probit 5, that is, 50% was found.

3.10 Median Lethal Concentration (LC_{50}) of Golan SL

Five different concentrations (0.5 ml/l, 1.0 ml/l, 1.5 ml/l, 2.0 ml/l and 2.5 ml/l) of Golan SL were applied to adult live bees in three cages (of 10 bees in each group). The number of bees with behavioral modifications or dead during 10, 20, 30, 40, 50 and 60 minutes was recorded. The percentage of bees that had died at each dose level were then transformed to probits in a probits table (Appendix 4). The results obtained were

used to plot a probit versus log-concentration Curve for Golan SL (Figure 5) and the concentration that would kill 50% of the bee population determined.

3.11 Calculation of Standard Error (S.E) for the Three Insecticides (Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL) Acute Toxicity

The S.E of the LC_{50} of the insecticides was calculated from the following formula of Ghosh (1984).

Approx S.E of $LC_{50} = \frac{\log LC^{84} - \log LC_{16}}{\sqrt{2N}}$. Where:

- ✓ LC_{84} and LC_{16} represent lethal concentrations at 84 and 16 respectively, meaning, the concentrations that will kill 84% and 16% respectively of the bee population determined.
- ✓ N is the number of honey bees in each group.

3.12 Statistical Analysis

The data collected were presented in tabular form, showing for each treatment group, as well as control group, the number of bees used and mortality at each observation time. The tables are found at the appendices. All observations (mortality data) were analysed using Microsoft Excel to generate the various curves with statistical equations where appropriate for data analysis. Specifically, data were analysed by tabulations and descriptive statistics of the Microsoft Excel output. Statistical Package for Social Sciences (SPSS) was also used to establish relationships between different concentrations. All statistical tests were estimated at 95% confidence level. Control mortality was made using Abbott‟s correction (Abbott, 1925).

CHAPTER FOUR

RESULTS

4.1 Commonly Used Insecticides in the Two Communities

From the interview conducted, the commonly sold insecticides in the area are Controller Super 2.5 EC, Pyrinex 48 EC, Goland SL, Pyrinex Quick 256 EC, Insector T. 45 and Sunhalothrin 2.5% EC. Controller Super 2.5 EC, Pyrinex 48 EC, and Goland SL were the most commonly used insecticides in the two communities studied. All the 40 farmers interviewed stated that they sprayed their crops with insecticides twice before harvesting in every planting season. The insecticides are used on crops such as maize, millet, rice, groundnuts, yams, cowpea and vegetables. Spraying was always done during flowering and fruiting. The targeted insects are usually caterpillars, beetles, aphids, moths, whiteflies, grasshoppers, crickets and locusts which feed on plants.

4.2 Observations Recorded During the Study

In the morning of the experiment, bees were found resting on the nylon mesh above the cages. When the insecticides were sprayed on them, they became aggressive and started flying restlessly in the cages. This occurred one to three minutes after spraying depending on the insecticide type and the concentration. All mortalities occurred after the bees fell from the nylon mesh and were crawling on the floor. Observations continued to the ninetieth minute but no recordings were made between 60 and 90 minutes since the control mortalities started occurring within that period. In some of the insecticide types and concentrations, all the bees were dead by the sixtieth minute.

4.3 LC_{50} of Controller Super 2.5 EC

The plot of probits versus log - concentration for calculation of LC_{50} for Controller Supper 2.5 is presented in Fig. 3.

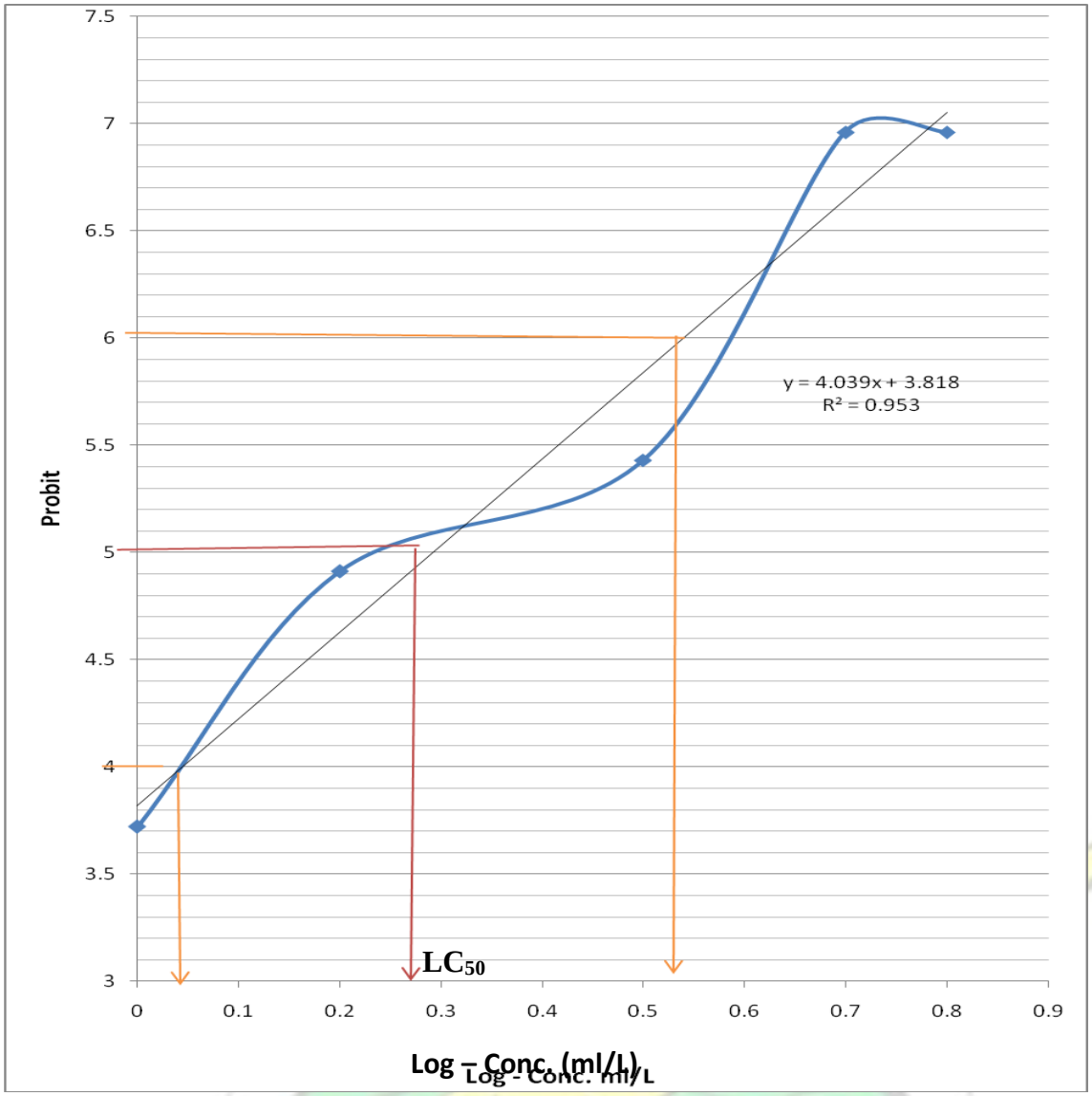

Fig. 3: Plot of probits versus log - concentration for calculation of LC50 for Controller Supper 2.5.

Log LC_{50} was found to be 0.27 and LC_{50} was 1.86 ml/L. The standard error of Controller Super 2.5 EC was calculated to be 0.53 using probit of 6 and a log concentration of 84 and probit of 4 and a log concentration of 16 from the plot of probits versus log - concentration for calculation of LC_{50}. The LC_{50} of Controller

Super 2.5 EC was 1.86 ± 0.53 with 95% confidence interval of 2.39 ml/L – 1.33 ml/L.

4.4 LC_{50} of Pyrinex 48 EC

The plot of probits versus log - concentration for the calculation of LC_{50} for Pyrinex 48 EC is given in Figure 4.

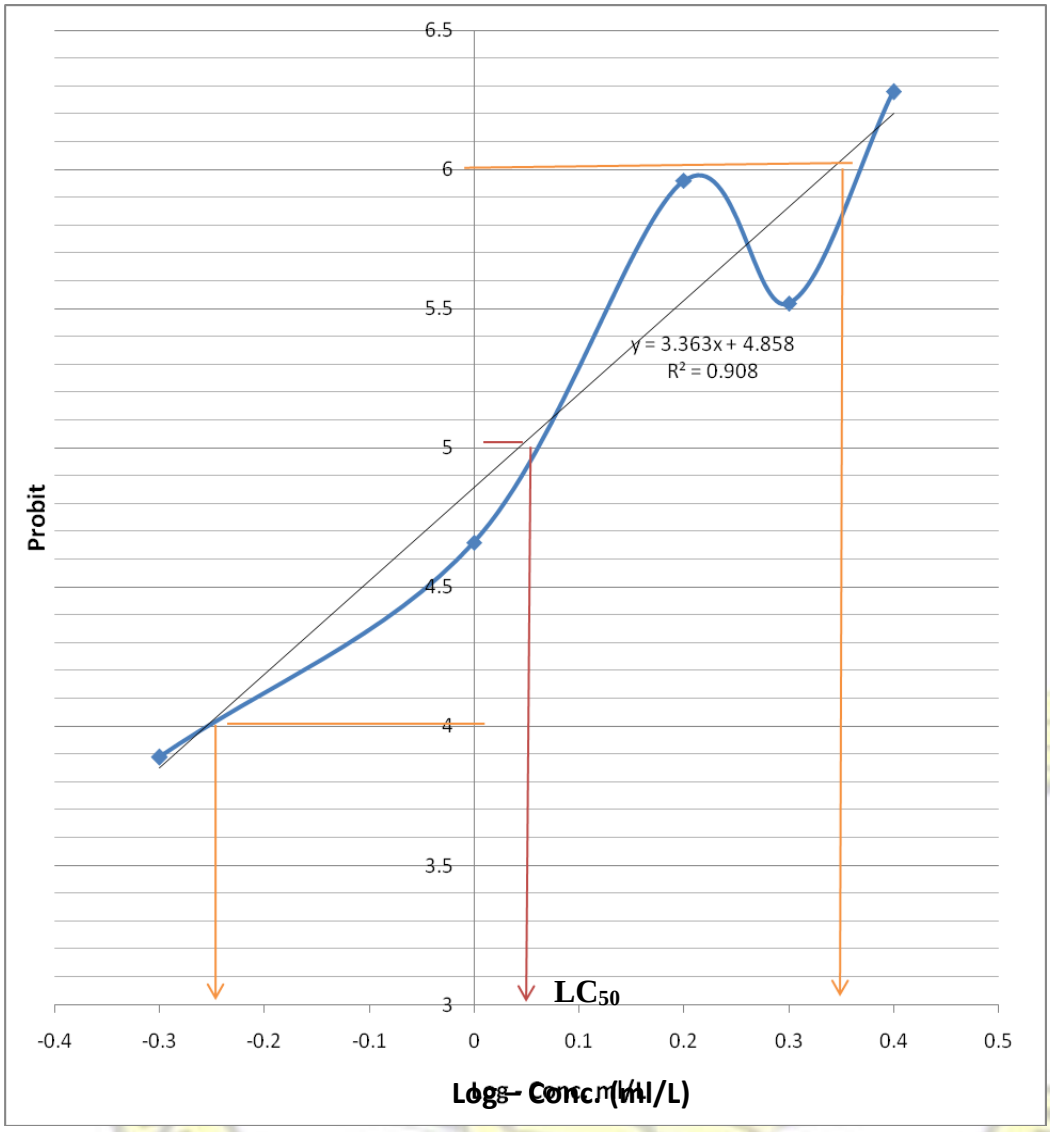

Fig. 4: Plot of probits versus log - concentration for calculation of LC_{50} for Pyrinex 48 EC.

In the case of Pyrinex 48 EC, Log LC_{50} was 0.04 and LC_{50} was 1.1 ml/L.

The standard error of Pyrinex 48 EC was 0.37 using the probits of 6 and log concentration of 84 and probit of 4 and log concentration of 16 from the plot of probits

versus log - concentration for the calculation of LC_{50}. LC_{50} of Pyrinex 48 EC was 1.1 ± 0.37, with 95% confidence interval of 1.47 - 0.73.

4.5 LC_{50} of Golan SL

The plot of probits versus log - concentration for the calculation of LC_{50} for Golan SL is shown in Figure 5.

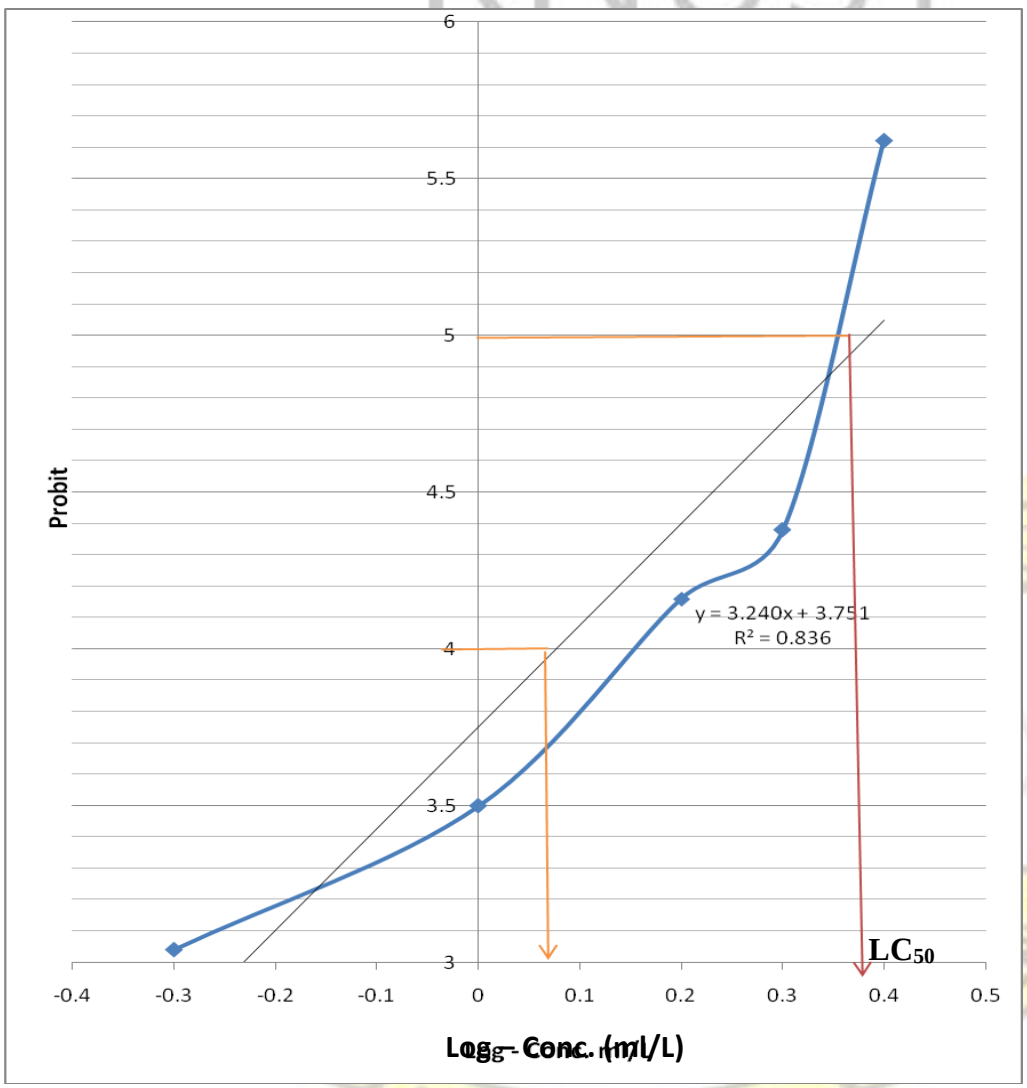

Fig. 5: Plot of probits versus log-concentration for calculation of LC_{50} for Golan SL.

Log LC_{50} of Golan SL was 0.39 and LC_{50} was 2.45 ml/L. The Standard Error was calculated to be 0.83 using the probit of 6 and log concentration of 84 and probit 4 and log concentration of 16 from the probits versus log-concentration curve. The LC_{50} of

Golan SL when applied was calculated to be 2.45 ± 0.83, with 95% confidence interval of 3.28 -1.62.

4.6 Mortality of Honey Bees after Exposure to the Different Concentrations of Controller Super 2.5 EC

The mean mortality of the honey bees after exposure to the different concentrations of Controller Super 2.5 EC for 60 minutes is given in Figure 6.

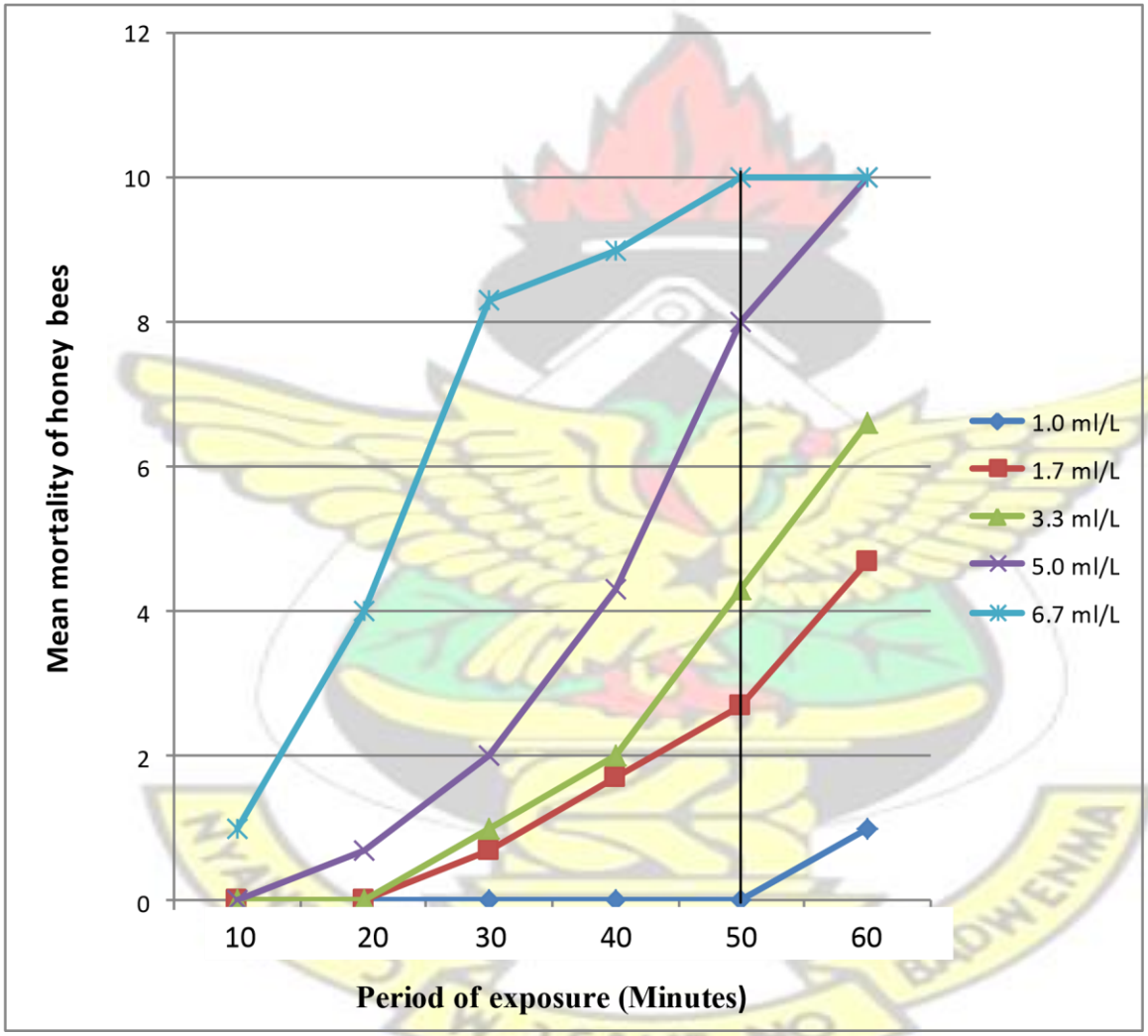

Fig 6: Mean mortality of Honey Bees after Exposure to the Different Concentrations of Controller Supper 2.5 EC for 60 minutes

Controller Supper 2.5 EC at a concentration of 6.7 ml/L gave the highest mortality (10 bees) at the 50th minute mark while the concentration of 1.0 ml/L recorded no mortality in the same 50th minute. Concentrations of 1.7 ml/L, 3.3 ml/L and 5.0 ml/l gave mean mortalities of 2.3, 4.3 and 8.0 respectively at the 50th minute (Fig. 6).

Results obtained from Pearson"s correlation analysis conducted confirmed there were close relationships between most of these concentrations. The concentration of 1.0 ml/L had significant correlations with the concentration at 1.7 ml/L ($r = 0.82$; $p = 0.04$). There was a significant relationship between concentration levels 1.7 ml/L and 3.3 ml/L ($r = 0.99$; $p = 0.00$) as well as 3.3 ml/L and 5.0 ml/L ($r = 0.82$; $p = 0.00$). There was significant difference ($p = 0.05$) between 5.0 ml/L and 6.7 ml/L. There was however, no significant difference between the lowest and highest concentrations of 1.0 ml/L and 6.7 ml/L ($p = 0.44$) (Table 9 in appendix 8).

4.6.2 Mortality of Honey Bees after Exposure to the Different Concentrations of Pyrinex 48 EC

The mean mortality of the honey bees after exposure to the different concentrations of Pyrinex 48 EC are given in Figure 7.

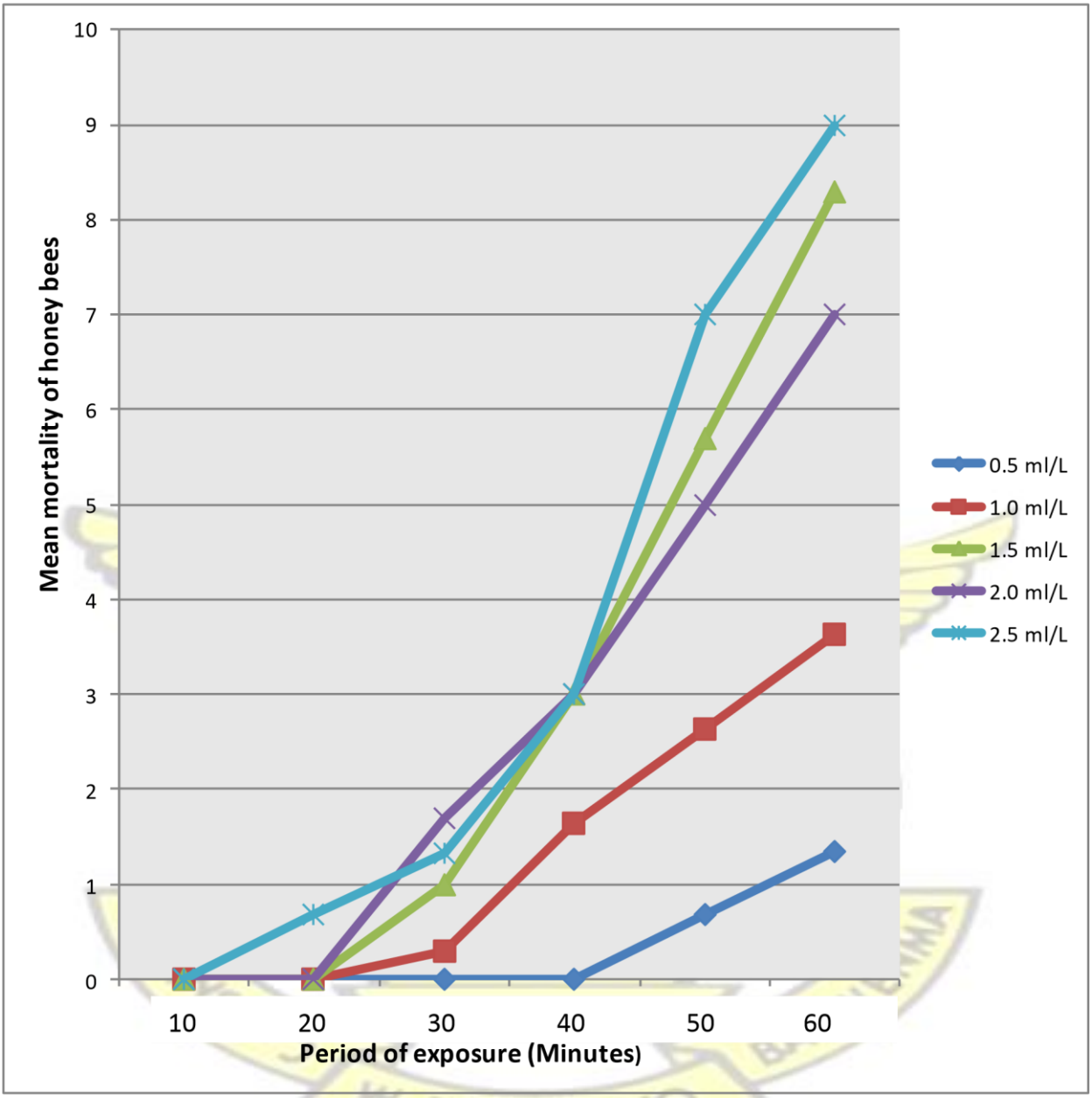

Fig 7: Mean mortality of Honey Bees after Exposure to the Different Concentrations of Pyrinex 48 EC for 60 minutes

Pyrinex 48 EC at a concentration of 2.5 ml/L gave the highest mortality (9 bees) at 60 minutes while the concentration of 0.5 ml/L gave the lowest mean mortality (1.3 bees). The concentrations of 1.0 ml/L, 1.5 ml/L and 2.0 ml/L gave mortalities of 3.7, 8.3 and 7.0 respectively.

Results from Pearson‟s correlation analysis demonstrated that there was a positive relationship (significant difference) between all the concentrations. Specifically, there was a significant difference between the concentration of 0.5 ml/L and 1.0 ml/L ($r = 0.90$; $p = 0.01$), 1.0 ml/L and 1.5 ml/L ($r = 0.99$; $p = 0.00$), 1.5 ml/L and 2.0 ml/L ($r = 0.99$; $p = 0.00$), 2.0 ml/L and 2.5 ml/L ($r = 0.99$; $p = 0.00$) as well as least and largest concentrations of 0.5 ml/L and 2.5 ml/L ($r = 0.94$; $p = 0.00$) (Table 10 in appendix 9).

4.6.3 Mortality of Honey Bees after Exposure to the Different Concentrations of Golan SL

The mean mortality of the honey bees after exposure to the different concentrations of Golan SL are indicated in Figure 8.

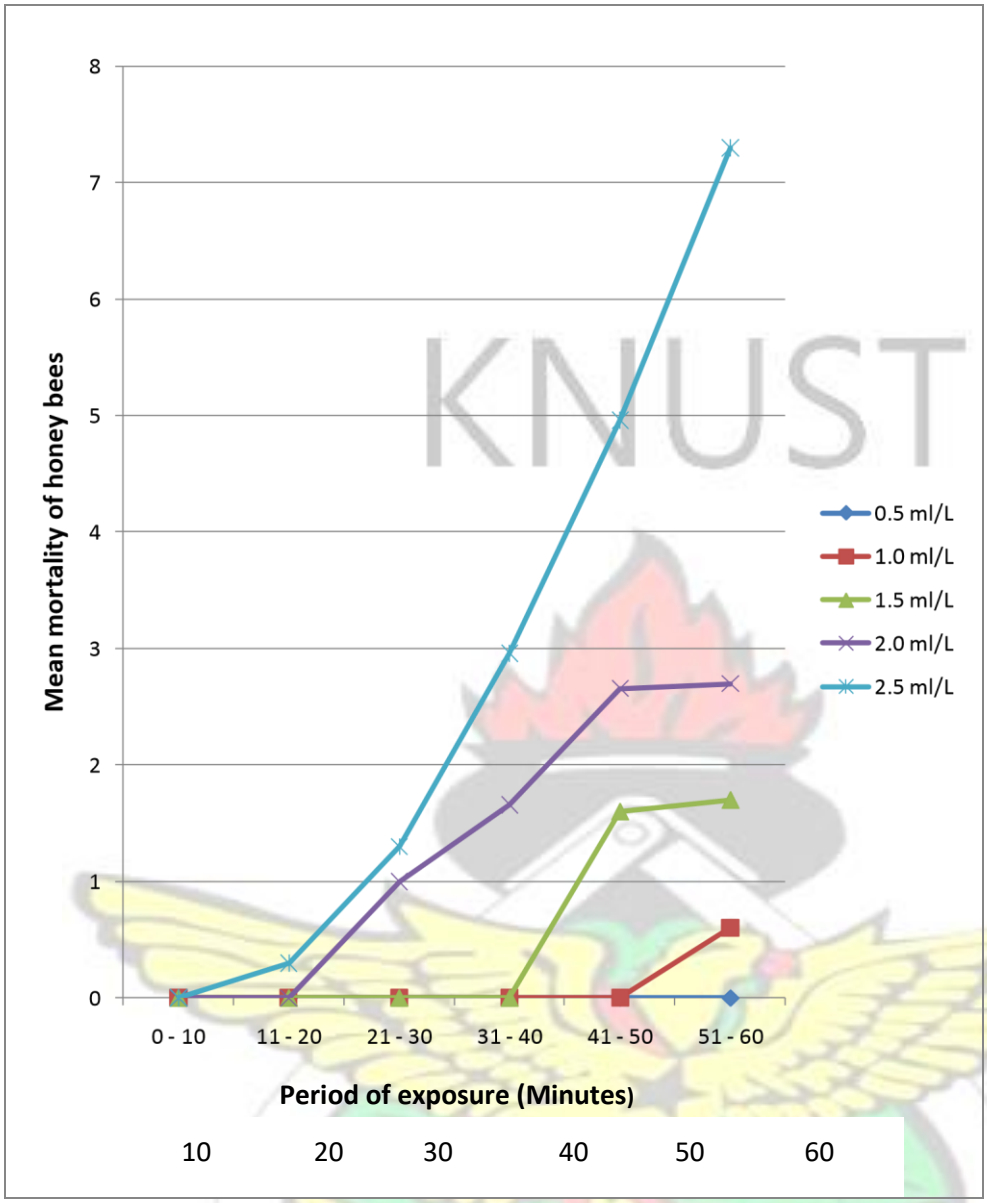

Fig 8: Mean mortality of Honey Bees after Exposure to the Different Concentrations of Golan SL for 60 minutes

Golan SL at a concentration of 2.5 gave the largest mortality (7.3 bees) at 60 minutes while the concentration of 0.5 recorded no mortality. Mean mortalities of 0.7, 1.7 and 2.7 were recorded at the concentrations of 1.0 ml/L, 1.5 ml/L and 2.5 ml/L respectively. The study further established association between the different concentrations levels recorded. Results demonstrated that concentration level at 0.5 ml/L could not be used to establish relationships because the concentration level showed no mortality at all.

However, the concentration level at 1.5 ml/L and 2.0 ml/L showed significant difference ($r = 0.84$; $p = 0.03$). There was a significant difference between the concentration levels 2.0 ml/L and 2.5 ml/L ($r = 0.95$; $p = 0.00$). Besides, there was no significant correlation between the concentration levels 1.0 ml/L and 1.5 ml/L ($r = 0.72$; $p = 0.11$), this can be found in Table 11 of appendix 10.

4.7.1 Toxicity of Controller Super 2.5 EC

The number of mortalities of honey bees after one (1) hour exposure to Controller Super 2.5 EC to Honey bees produced a toxicity curve (Figure 9) with the equation: y = 1.5254x + 1.0987. Where „Y" is the number of mortalities in bees and „X" is the concentration of Controller Super 2.5 EC per litre of water. If the „X" value kills many bees, it means the concentration is more toxic.

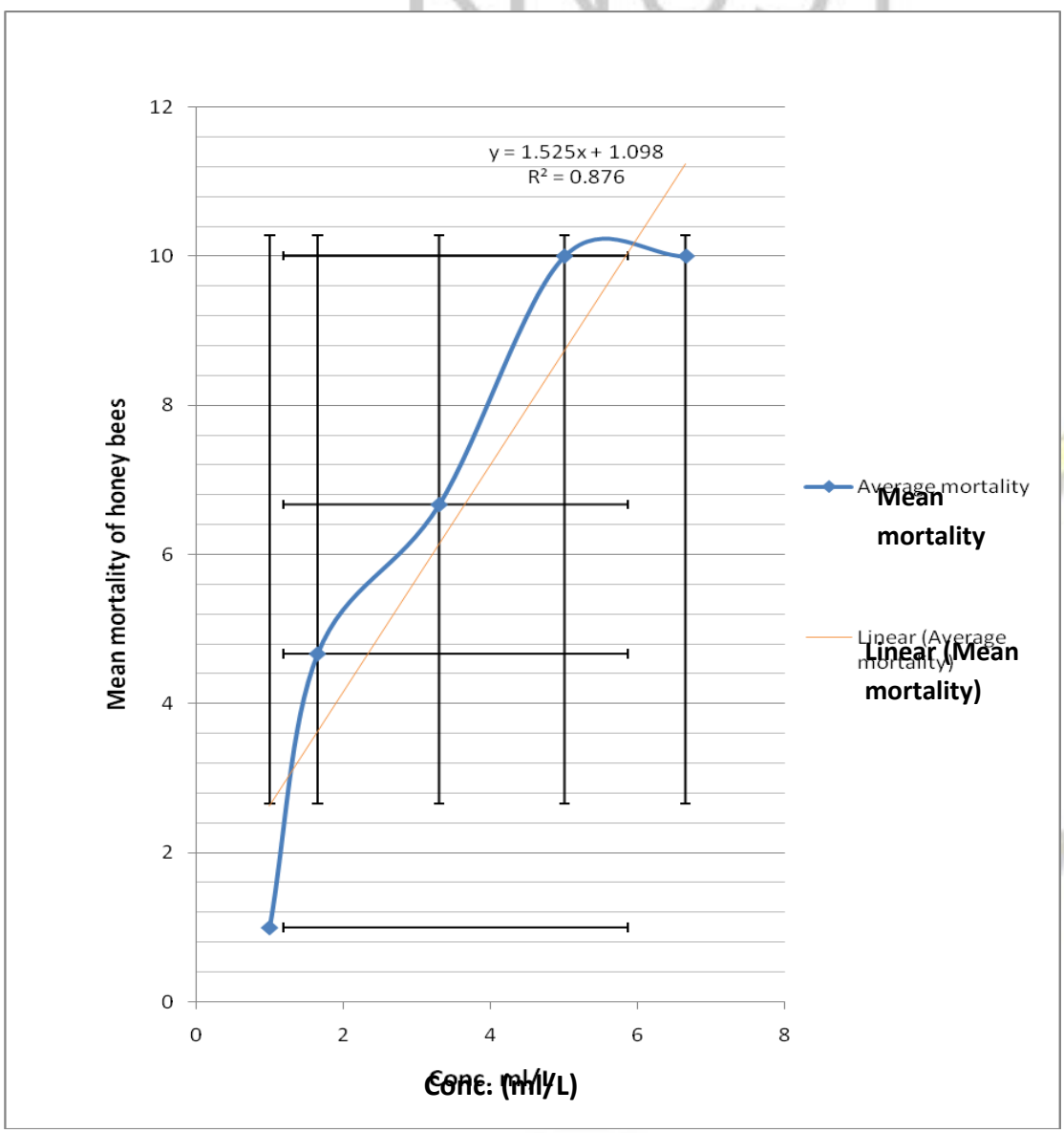

Fig 9: Toxicity of Controller Super 2.5 EC due to one hour exposure to honey bees

The toxicity curve presented in figure 9 showed the recommended level of Controller Super 2.5 EC.

4.7.2 Toxicity of Pyrinex 48 EC

The number of mortalities of honey bees after one (1) hour exposure to Pyrinex 48 EC produced a toxicity curve (Figure 10) with the equation y = 3.734x + 0.265.

Where „Y" is the number of mortalities in bees and „X" is the concentration of Pyrinex 28 EC. If the „X" value kills so many bees, it means that the concentration is more toxic to the bees.

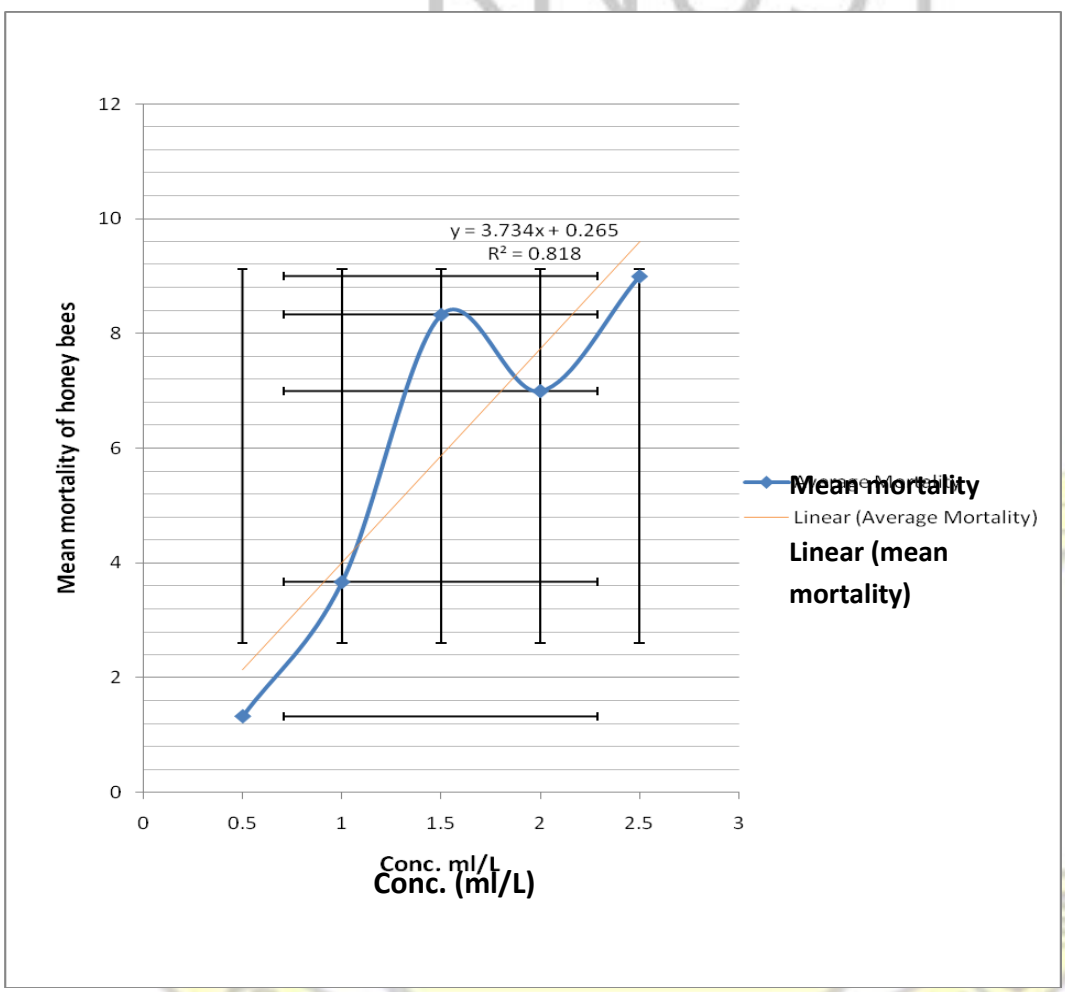

Fig 10: Toxicity of Pyrinex 48 EC due to one hour exposure to honey bees.

The toxicity curve presented in figure 10 showed the recommended level of Pyrinex 48 EC.

4.7.3: Toxicity of Golan SL

The number of mortalities of honey bees after one (1) hour exposure to Golan SL produced a toxicity curve (Figure 11) with the equation: y = 3.332x - 2.464. Where „Y" is the number of mortalities in bees and „X" is the concentration of Golan SL. If the „Y" value is large, the concentration is more toxic.

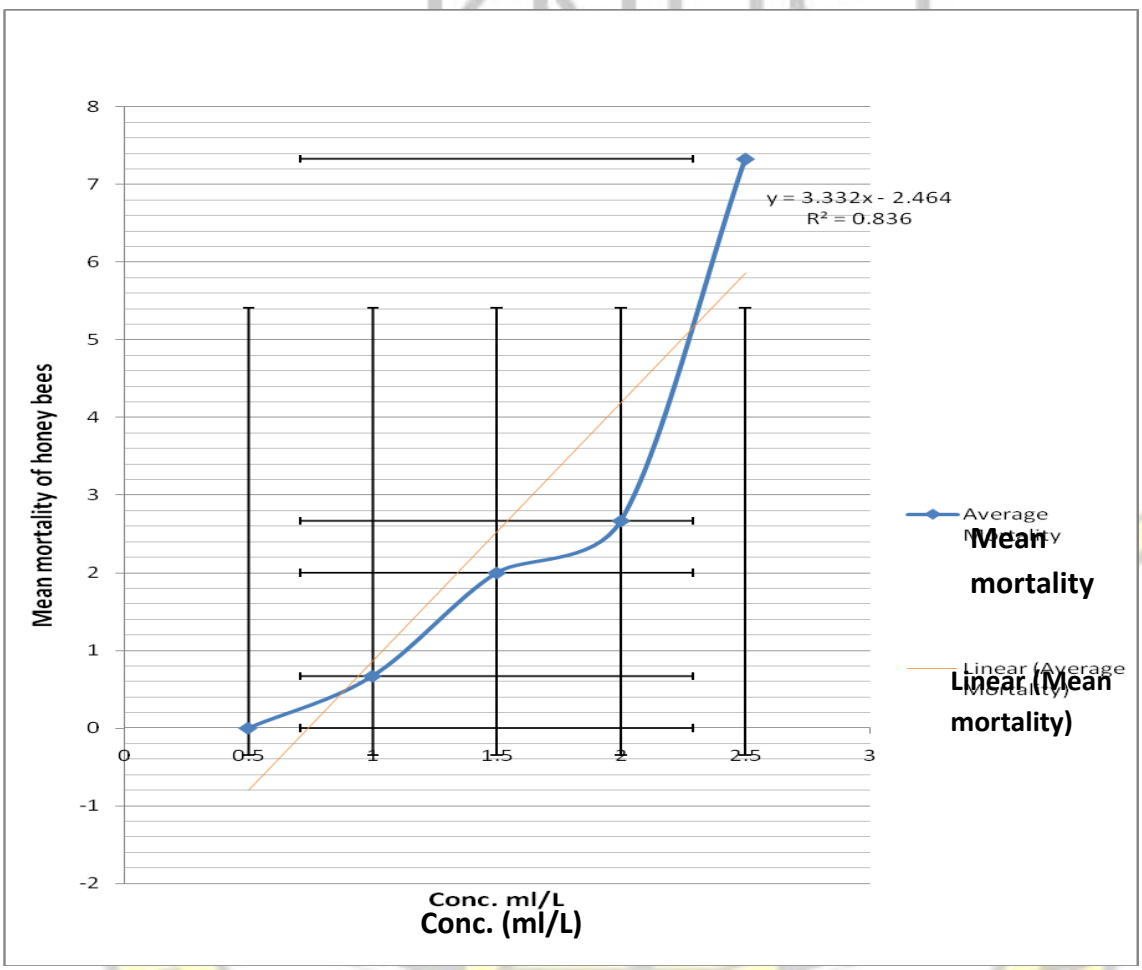

Fig. 11: Toxicity of Golan SL due to one hour exposure to honey bees.

The toxicity curve presented in figure 11 showed the recommended level of Golan SL.

CHAPTER FIVE

DISCUSSION

5. 1 Use of insecticides in the study area

The oral interview conducted in the study area showed that the commonly used insecticides were Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL. The people explained that the insecticides were effective in controlling pests, were less expensive compared to other types of insecticides and were always available and accessible to farmers. The variation of the toxicity of these insecticides to honey bees may be due to the active ingredients they contain and the concentrations administered.

The likelihood of exposure of honey bees to the insecticides could occur when honey bees living near agricultural fields go foraging on food crops sprayed with the insecticides.

5.2 The LC_{50} of the Three Insecticides (Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL

The calculated median lethal concentrations (LC_{50}) of Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL were 1.86, 1.10 and 2.45 respectively. In general, the smaller the LC_{50} / LD_{50} value, the more toxic the chemical is. The opposite is also true: the larger the LC_{50} / LD_{50} value the lower the toxicity. Pyrinex 48 EC was the most toxic with an LC_{50} of 1.1 ± 0.37 ml/L, with 95% confidence interval of 1.47 to 0.73. Controller Super 2.5 EC demonstrated a similar level of toxicity to the Pyrinex 48 EC with an LC_{50} value of 1.86 ± 0.53 ml/L at 95% confidence interval of 2.39 – 1.33 ml/L. Golan SL was the least toxic among the three insecticides under consideration with LC_{50} value of 2.45 ± 0.83 ml/L, at 95% confidence interval of 3.28 - 1.62 ml/L. The differences in the LC_{50} values of the insecticides might be due to the type of active ingredients they contained and the concentrations administered.

Controller Super 2.5 EC contained Lambda-cyhalothrin; Pyrinex 48 EC contained Chlorpyrifos 480 GR/LT (O, O-Diethyl O-3, 5-6-trichloro-2-pyridyl

phosphorothioate) and Golan SL contained acetamiprid. These active ingredients are toxic to insects but vary in toxicity. In the present case, Chlorpyrifos 480 GR/LT (O, O-Diethyl O-3, 5-6-trichloro-2-pyridyl phosphorothioate) in Pyrinex 48 EC was the most toxic and acetamiprid in Golan SL the least toxic. The concentrations administered were also important in determining the toxicity (LC_{50}) since the results showed significant differences between each concentration and the subsequent one. In the case of Controller Super 2.5 EC, there was significant difference in each concentration level and the subsequent one with „p" values of 0.04, and 0.00. However, there was no significant difference between the lowest and highest concentration levels of 1.0 ml/L and 6.7 ml/L (p = 0.44). This result is found in table
9 of appendix 8.

Pyrinex 48 EC showed similar results as was found in controller super 2.5 EC. There was significant difference between all concentration levels wch gave „p" values of 0.01 and 0.00. The lowest and highest concentrations of 0.5 ml/L and 2.5 ml/L also showed a positive correlation with p = 0.00.

Goland SL showed positive correlation such that there was significant difference between most of the concentration levels with „p" values of 0.03 and 0.00. Besides, there was no significant difference between concentrations 1.0 ml/L and 1.5 ml/L with p = 0.11. However, 0.5 ml/L could not be used to establish relationships because the concentration level produced zero mortality at all the time recorded (Table 11 in appendix 10). The overall results from Pearson"s correlation analyses showed that the various concentrations used to determine the acute toxicity (LC_{50}) of the three insecticide brands were significantly related to each other.

5.3 Mortality of Honey Bees after Exposure to the Three Insecticides at Different Concentrations

Mortality of bees due to exposure to the different concentrations of all the three insecticides (Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL) was higher than the control. Generally as the concentration of the three insecticides increased, there was a corresponding increase in mortality of the bees. The highest concentrations of 6.7, 2.5 and 2.5 milliliters per liter of water for Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL respectively used resulted in mean mortalities of 10.0, 9.0 and 7.3 respectively, after 60 minutes of exposure. However, the lowest concentrations of 1.0 ml/L, 0.5 ml/L, and 0.5 ml/L resulted in the mean mortalities of 1.0, 1.3 and zero respectively in the 60th minute.

At the recommended formulation of 1.0 ml/L for Controller Super 2.5 EC, no mortality was recorded at the 50th minute while at 6.7 ml/l all the ten bees died at the 50th minute (Fig. 6). However the recommended formulation of 1.0 ml/L produced some mortality (mean mortality of 1.3 bees) at the 60th minute. This might be due to the fact that the bees had to take in greater quantity of the insecticide before they could show any toxic signs. The concentration of 1.7 ml/L showed mean mortality of 0.7 bees in the 30th minute which increased steadily to 4.7 bees in the 60th minute.

There was significant difference ($p = 0.04$) between 1.0 ml/L and 1.7 ml/L.
Concentration of 3.3 ml/L gave the same mortality pattern as the 1.7 ml/L except that it showed a greater mean mortality (6.3 bees) in the 60th minute while the 1.7 ml/L gave mean mortality of 4.7 bees in the 60th minute. There was significant difference ($p = 0.00$).

Concentration 5.0 ml/L also showed some mortality (0.3 bees) in the 20th minute which increased steadily to 10 at the 60th minute where all the bees died. There was significant difference (p = 0.04) between 3.3 ml/L and 5.0ml/L. The 6.7 ml/L concentration showed some mortality of 0.7 bees in the 10th minute which increased to 8.3 bees in the 30th minute. This might be that the active ingredients at that concentration exhibited greater toxicity after few minutes of exposure leading to death of the bees. All 10 bees were dead by the 50th minute. There was significant difference (p = 0.05) between 5.0 ml/L and 6.7 ml/L (Table 9 in appendix 8). The overall mean mortality recorded for the concentrations 1.0 ml/L, 1.7 ml/L, 3.3 ml/L,

5.0 ml/L and 6.7 ml/L were 1.3, 4.7, 6.7, 10 and 10 respectively at the 60th minute.

There were positive correlations among the various concentrations which can be found in table 9 of appendix 8. This result is similar to the findings of Penelope *et al.*, (2012) in Scotland that when colonies of bumble bees were exposed to recommended formulations of different concentrations of imidacloprid, it caused some mortality. It showed that bumble bees, which are wild pollinators, were suffering similar impacts of pesticide exposure to "managed" honey bees.

The recommended formulations of 0.5 ml/L for Pyrinex 48 EC showed mortality (mean mortality of 0.7 bees) at the 50th minute which increased steadily to the 60th minute (Fig. 7). Similarly, the recommended formulation of 1.0 ml/L produced some mortality (mean mortality of 0.3 bees) at the 30th minute and steadily increased to the 60th minute while concentration 2.0 ml/L produced mean mortality of 7.0 bees at the 60th minute. This might be that the toxic chemicals at the concentration 1.5 ml/L exhibited greater toxicity to the bees than the 2.0 ml/L during the exposure leading to

more mortality of the bees (Fig. 7). Other possible reasons for the bees mortality includes stress since the bees were caught from their hives and transported to different location where they were caged for the study. Variations in environmental conditions such as temperature, relative humidity and light at the study site could differ from what existed in the hive which might have also contributed to bees‟ mortality. The time and period of capture and exposure to the various insecticides could also affect the health of the bees leading to their mortality.

The 2.5 ml/L produced some mortality of 0.7 bees in the 10th minute which showed a slow increase to the 40th minute (mean mortality of 3.0 bees) but increased steadily to the 60th minute. The overall mean mortality recorded for the concentrations; 0.5 ml/L, 1.0 ml/L, 1.5 ml/L, 2.0 ml/L and 2.5 ml/L were 1.3, 3.7, 8.3, 7.0 and 9.0 respectively at the 60th minute (Fig. 7). Results from Pearson‟s correlation analysis showed significant difference among the various concentrations. This is found in Table 10 of appendix 9.

At the recommended formulation of 0.5 ml/L for Golan SL, no mortality (mean mortality of 0.0 bees) was recorded at the 60th minute while at 2.5 ml/L seven bees died at the 60th minute (Fig. 8). This could be due to the fact that the active ingredients in the 0.5 ml/L concentration were too low to cause any mortality in the bees even after longer period of exposure. The concentrations of 1.0 ml/L, 1.5 ml/L and 2.0 ml/L showed similar mortality pattern of 0.7, 1.7 and 2.7 bees in the 60th minute. The mortality generally increased with increasing concentrations and longer period of exposure. The 2.5 ml/L concentration showed some mortality of 0.3 bees in the 10th minute which increased steadily to 7.3 bees in the 60th minute. The overall mean mortality recorded

for the concentrations 0.5 ml/L, 1.0 ml/L, 1.5 ml/L, 2.0 ml/L and 2.5 ml/L were 0.0, 0.7 , 1.7, 2.7 and 7.3 respectively at the 60th minute (Fig. 8).

The study further established correlations between the different concentrations which showed significant difference between most of the concentration levels applied. Results demonstrated that concentration of 0.5 ml/L could not be used to show relationships because the concentration level did not record mortality throughout the period of exposure. This could be that the active ingredients in the concentration were too low to cause any mortality even with longer period of exposure (Table 11 in appendix 10).

The concentrations administered were important in determining the mortality of honey bees since each concentration level showed a significant difference to the subsequent one in all the three insecticide brands.

5.4 Impact of Recommended Levels of Insecticides Applied to Crops on Honey Bees

The recommended levels for application of Pyrinex 48 EC and Controller Super 2.5 EC on maize are 100 ml per 100 Liters of water and 100 ml per 15 Liters of water respectively. Given the toxicity curves of Pyrinex 48 EC and Controller super 2.5 EC (as $y = 3.734x + 0.265$ and $y = 1.5254x + 1.0987$ respectively), (Figs. 10 and 9 respectively), honey bees which would be inadvertently exposed to the maize for 1 hour during or after the application of Pyrinex 48 EC will cause mean mortality of 4 bees out of every 10 bees (Fig. 10). However, when the Controller Super 2.5 EC is applied at the recommended concentration (100mls per 15 litres of water) on maize, it will cause total mortality within 1 hour. The recommended formulation concentration for Golan SL is

30 ml per 100 Liters of water on vegetables. This would show no mortality when honey bees are inadvertently exposed to it within 1 hour of application (Fig. 11).

Given the toxicity curves of Pyrinex 48 EC and Golan SL (as y = 3.734x + 0.265 and y = 3.332x - 2.464 respectively) (Figs. 10 and 11 respectively), after one (1) hour of exposure, the Pyrinex 48 EC will cause mean mortality of 2.1 out of every ten honey bees while Golan SL will show no mortalities.

The findings generally agreed with that of Palmer *et al*. (2007) that pesticide use reduces biodiversity, contributes to pollinator decline, destroys habitat and threatens endangered species. The use of agricultural chemicals can have damaging effects on honey bees.

This has been stated by Feldman (2011) that crop farmers who depend on honey bees for the pollination of their crop(s) must constantly maintain a delicate balance between protecting their crops from pests and pathogens, and protecting the insects that are necessary to pollinate these crops.

CHAPER SIX

CONLUSION AND RECOMMENDATIONS

6.1 Conclusion

The interview results showed that farmers in the study area were most likely to continue using insecticides for the control of pests to ensure higher crop yield. The LC_{50} for the three insecticides used were within the recommended concentrations provided on the labels of the various bottles of the insecticides. The overall result of the present study clearly demonstrated that mortalities occurred when honey bees were exposed to

different concentrations of all the three insecticides at the manufacturers‟ recommended concentrations. However, higher mortalities occurred at higher concentrations and longer period of exposure. It can therefore be concluded that the use of these insecticides continue to kill bees and reduce pollinator population.

6.2 Recommendations

It is recommended that:

1. Further studies should be carried out on the effects of chemical insecticides on honey bees.
2. Regulatory processes for registration and labeling of insecticides for agricultural use should be based on testing and labeling according to our prevailing conditions such as temperature, humidity and light in Ghana rather than studies carried in other countries and the science of the past decades.
3. The use of contact insecticides as well as agro-chemicals (insecticides) should be reduced on bee-pollinated crops during crop flowering.
4. List of insecticides that are safe for use in combinations should be made available to Agric Extension Officers to educate the local farmers.
5. There should be better and frequent communication between industry, academia and government on the save use of pesticides in the environment, this will help to ensure better risk assessment.

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APPENDICES

Appendix 1: Mortality Observation of honey bees when exposed to the Control Experiment (1L of distilled Water)

Table 2: Mortality of honey bees after exposure to control (distill water)

TIME (Minutes)	Mortality Observation of Control Experiment			Mean mortality
	Box 1	Box 2	Box 3	
1 to 10 min	0.0	0.0	0.0	**0.0**
11 to 20 min	0.0	0.0	0.0	**0.0**
21 to 30 min	0.0	0.0	0.0	**0.0**
31 to 40 min	0.0	0.0	0.0	**0.0**
41 to 50 min	0.0	0.0	0.0	**0.0**
51 to 60 min	0.0	0.0	0.0	**0.0**
61 to 70 min	1.0	0.0	0.0	**0.3**
71 to 80 min	1.0	0.0	1.0	**0.7**
81 to 90 min	0.0	1.0	1.0	**0.7**
91 to 100 min	1.0	1.0	1.0	**1.0**

Appendix 2: Mortality evolution of honey bees when exposed to different doses of Controller Super 2.5 EC

Table 3: Mortality of honey bees after exposure to different concentrations of Controller Super 2.5 EC

CONCENTRATION	TIME	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
1.0 ml/L	Box 1	0.0	0.0	0.0	0.0	0.0	2.0
	Box 2	0.0	0.0	0.0	0.0	0.0	1.0
	Box 3	0.0	0.0	0.0	0.0	0.0	0.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**
1.7 ml/L	Box 1	0.0	0.0	1.0	3.0	0.0	5.0
	Box 2	0.0	0.0	1.0	0.0	2.0	4.0
	Box 3	0.0	0.0	0.0	1.0	3.0	5.0
	Average	**0.0**	**0.0**	**0.2**	**1.0**	**1.7**	**4.7**
3.3 ml/L	Box 1	0.0	0.0	1.0	2.0	5.0	7.0
	Box 2	0.0	0.0	1.0	3.0	5.0	7.0
	Box 3	0.0	0.0	1.0	0.0	3.0	6.0
	Average	**0.0**	**0.0**	**1.0**	**1.7**	**4.3**	**6.7**
5.0 ml/L	Box 1	0.0	1.0	3.0	5.0	9.0	10.0
	Box 2	0.0	1.0	2.0	5.0	8.0	10.0
	Box 3	0.0	0.0	1.0	3.0	7.0	10.0
	Average	**0.0**	**0.2**	**2.0**	**4.3**	**8.0**	**10.0**
6.7 ml/L	Box 1	2.0	5.0	9.0	9.0	10.0	10.0
	Box 2	1.0	4.0	9.0	10.0	10.0	10.0
	Box 3	0.0	3.0	7.0	8.0	10.0	10.0
	Average	**1.0**	**4.0**	**8.3**	**9.0**	**10.0**	**10.0**

Appendix 3: Mortality evolution of honey bees when exposed to different doses of Pyrinex 48 EC

Table 4: Mortality of honey bees after exposure to different concentrations of Pyrinex 48 EC

Concentration	TIME	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
0.5 ml/L	Box 1	0.0	0.0	0.0	0.0	1.0	2.0
	Box 2	0.0	0.0	0.0	0.0	1.0	1.0
	Box 3	0.0	0.0	0.0	0.0	0.0	1.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.7**	**1.3**
1.0 ml/L	Box 1	0.0	0.0	1.0	2.0	3.0	3.0
	Box 2	0.0	0.0	0.0	2.0	3.0	5.0
	Box 3	0.0	0.0	0.0	1.0	2.0	3.0
	Average	**0.0**	**0.0**	**0.3**	**1.6**	**2.7**	**3.7**
1.5 ml/L	Box 1	0.0	0.0	2.0	4.0	7.0	9.0
	Box 2	0.0	0.0	1.0	3.0	5.0	8.0
	Box 3	0.0	0.0	0.0	2.0	5.0	8.0
	Average	**0.0**	**0.0**	**1.0**	**3.0**	**5.7**	**8.3**
2.0 ml/L	Box 1	0.0	0.0	2.0	4.0	6.0	9.0
	Box 2	0.0	0.0	2.0	3.0	5.0	7.0
	Box 3	0.0	0.0	1.0	2.0	4.0	5.0
	Average	**0.0**	**0.0**	**1.7**	**3.0**	**5.0**	**7.0**
2.5 ml/L	Box 1	0.0	1.0	2.0	3.0	8.0	10.0
	Box 2	0.0	1.0	1.0	3.0	7.0	9.0
	Box 3	0.0	0.0	1.0	3.0	6.0	8.0
	Average	**0.0**	**0.7**	**1.3**	**3.0**	**7.0**	**9.0**

Appendix 4: Mortality evolution of honey bees when exposed to different doses of Golan SL

Table 5: Mortality of honey bees after exposure to different concentrations of Golan SL

Concentration	TIME	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
0.5 ml/L	Box 1	0.0	0.0	0.0	0.0	0.0	0.0
	Box 2	0.0	0.0	0.0	0.0	0.0	0.0
	Box 3	0.0	0.0	0.0	0.0	0.0	0.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
1.0 ml/L	Box 1	0.0	0.0	0.0	0.0	0.0	1.0
	Box 2	0.0	0.0	0.0	0.0	0.0	0.0
	Box 3	0.0	0.0	0.0	0.0	0.0	1.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.7**
1.5 ml/L	Box 1	0.0	0.0	0.0	0.0	2.0	2.0
	Box 2	0.0	0.0	0.0	0.0	1.0	1.0
	Box 3	0.0	0.0	0.0	0.0	1.0	2.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**1.3**	**1.7**
2.0 ml/L	Box 1	0.0	0.0	2.0	2.0	3.0	3.0
	Box 2	0.0	0.0	1.0	2.0	3.0	3.0
	Box 3	0.0	0.0	0.0	1.0	2.0	2.0
	Average	**0.0**	**0.0**	**1.0**	**1.7**	**2.7**	**2.7**
2.5 ml/L	Box 1	0.0	1.0	2.0	4.0	6.0	8.0
	Box 2	0.0	0.0	1.0	2.0	4.0	7.0
	Box 3	0.0	0.0	1.0	3.0	5.0	7.0
	Average	**0.0**	**0.3**	**1.3**	**3.0**	**5.0**	**7.3**

Appendix 5: Concentration - Response Values for Controller Super 2.5 EC

Table 6: Concentration - Response Values for Controller Super 2.5 EC

CONC. GROUP	CONCENTRATION ml/L	LOG - CONCENTRATION	MEAN MORTALITY	PERCENTAGE MORTALITY	CORRECTED PERCENTAGE	PROBIT
1.	1.0	0.0	1.0	10.0	10.0	3.72
2.	1.7	0.2	4.7	46.7	46.7	4.91
3.	3.3	0.5	6.7	66.7	66.7	5.43
4.	5.0	0.7	10.0	100.0	97.5	6.96
5.	6.7	0.8	10.0	100.0	97.5	6.96

Appendix 6: Concentration - Response Values for Pyrinex 28 EC

Table 7: Concentration - Response Values for Pyrinex 48 EC

CONC.GROUP	CONCENTRATION ml/L	LOG - CONCENTRATION	MEAN MORTALITY	PERCENTAGE MORTALITY	CORRECTED PERCENTAGE	PROBIT
1.	0.5	-0.3	1.3	13.3	13.3	3.89
2.	1.0	0.0	3.7	36.7	36.7	4.66
3.	1.5	0.2	8.3	83.3	83.3	5.96
4.	2.0	0.3	7.0	70.0	70.0	5.52
5.	2.5	0.4	9.0	90.0	90.0	6.28

Appendix 7: Concentration - Response Values for Golan SL

Table 8: Concentration - Response Values for Golan SL

CONC. GROUP	CONCETRATION ml/L	LOG - CONCENTRATION	MEAN MORTALITY	PERCENTAGE MORTALITY	CORRECTED PERCENTAGE	PROBIT
1.	0.5	-0.3	0.0	0.0	2.5	3.04
2.	1.0	0.0	0.67	6.7	6.7	3.50
3.	1.5	0.2	2.0	20.0	20.0	4.16
4.	2.0	0.3	2.7	26.7	26.7	4.38
5.	2.5	0.4	7.3	73.3	73.3	5.62

Source: Authors construct from Laboratory Experiment 2013 Appendix 8: Pearson's Correlations between different concentration Levels

(Controller Super 2.5 EC).

Table 9: Pearson"s Correlations between different concentration Levels (Controller Super 2.5 EC)

Concentration Level		*1.0 ml/L*	*1.7 ml/L*	*3.3 ml/L*	*5.0 ml/L*	*6.7 ml/L*
1.0 ml/L	Pearson Correlation	1	.821*	.795	.702	.390
	Sig. (2-tailed)		.045	.059	.120	.445
	N	6	6	6	6	6
1.7 ml/L	Pearson Correlation	**.821***	1	.994**	.979**	.767
	Sig. (2-tailed)	**.045**		.000	.001	.075
	N	**6**	6	6	6	6
3.3 ml/L	Pearson Correlation	.795	**.994****	1	.988**	.763
	Sig. (2-tailed)	.059	**.000**		.000	.078
	N	6	**6**	6	6	6

5.0 ml/L	Pearson Correlation	.702	.979**	**.988****	1	.820*
	Sig. (2-tailed)	.120	.001	**.000**		.046
	N	6	6	**6**	6	6
6.7 ml/L	Pearson Correlation	**.390**	.767	.763	**.820***	1
	Sig. (2-tailed)	**.445**	.075	.078	**.046**	
	N	**6**	6	6	**6**	6

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 9: Pearson's Correlations between different concentration Levels (Pyrinex 48 EC)

Table 10: Pearson"s Correlations between different concentration Levels (Pyrinex 48 EC)

Concentration Level		***0.5 ml/L***	***1.0 ml/L***	***1.5 ml/L***	***2.0 ml/L***	***2.5 ml/L***
0.5 ml/L	Pearson Correlation	1	.907*	.939**	.907*	.942**
	Sig. (2-tailed)		.013	.006	.013	.005
	N	6	6	6	6	6
1.0 ml/L	Pearson Correlation	**.907***	1	.995**	.987**	.986**
	Sig. (2-tailed)	**.013**		.000	.000	.000
	N	**6**	6	6	6	6
1.5 ml/L	Pearson Correlation	.939**	**.995****	1	.993**	.994**
	Sig. (2-tailed)	.006	**.000**		.000	.000
	N	6	**6**	6	6	6

	Pearson Correlation	.907*	.987**	**.993****	1	.984**
2.0 ml/L	Sig. (2-tailed)	.013	.000	**.000**		.000
	N	6	6	**6**	6	6
	Pearson Correlation	**.942****	.986**	.994**	**.984****	1
2.5 ml/L	Sig. (2-tailed)	**.005**	.000	.000	**.000**	
	N	**6**	6	6	**6**	6

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 10: Pearson's Correlations between different concentration Levels (Pyrinex 48 EC)

Table 11: Pearson‟s Correlations between different concentration Levels (Golan SL)

		0.5 ml/L	*1.0 ml/L*	*1.5 ml/L*	*2.0 ml/L*	*2.5 ml/L*
	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]
0.5 ml/L	Sig. (2-tailed)	.	.	.	.	.
	N	6	6	6	6	6
	Pearson Correlation	.[a]	1	.716	.539	.748
1.0 ml/L	Sig. (2-tailed)	.		.109	.269	.087
	N	6	6	6	6	6
	Pearson Correlation	.[a]	**.716**	1	.847*	.919**
1.5 ml/L	Sig. (2-tailed)	.	**.109**		.033	.009
	N	6	**6**	6	6	6
	Pearson Correlation	.[a]	.539	**.847***	1	.956**
2.0 ml/L	Sig. (2-					

	tailed)	.	.269	**.033**		.003
	N	6	6	**6**	6	6
	Pearson Correlation	.[a]	.748	.919**	**.956****	1
2.5 ml/L	Sig. (2-tailed)	.	.087	.009	**.003**	
	N	6	6	6	**6**	6

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Appendix 11: Transformation of percentages to probits

Table 12: Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	----	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.20	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
---	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Source; http://userwww.sfsu.edu/efc/classes/biol710/probit/ProbitAnalysis.pdf

Appendix 12: Interview Guide for Common Insecticides Usage

1. What are the some of the insecticides that are available in your local market?
2. Which of the insecticides do you commonly used?
3. How often do you apply each of your commonly preferred insecticides?
4. Why do you commonly prefer to use those insecticides and not the others?