KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCES

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

ECOLOGICAL IMPLICATIONS OF PEST MANAGEMENT IN A FOREST AGROECOSYSTEM: A CASE STUDY OF THE RELATIVE TOXICITY OF SOME PESTICIDES USED FOR THE CONTROL OF PESTS IN AKUMADAN, GHANA

A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE)

SEFA PEPRAH

BA (HONS) GEOGRAPHY

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DECLARATION

I hereby declare that, this thesis presented to the Department of Theoretical and Applied Biology, in partial fulfillment for the award of MSc. Degree, is a true account of the student's own work.

SEFA PEPRAH ………………………….. ……………………….
(STUDENT) SIGNATURE DATE

PROF. K. YEBOAH-GYAN …………………………….. ……………………….
(SUPERVISOR) SIGNATURE DATE

DR. P. K. BAIDOO ………………………….. ……………………….
(HEAD OF DEPT.) SIGNATURE DATE

DEDICATION

To the Glory of God, this thesis is dedicated to my family.

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ABSTRACT

The use of pesticides on agricultural lands to protect crops and agricultural produce has increased worldwide since the advent of the 'green revolution'. In Ghana pesticide use continues to increase as agricultural production intensifies highlighting the potential problems in conserving biodiversity in agricultural ecosystems. Available literature supports the fact that a lot of research work has been done on the toxicity of pesticides in agroecosystems elsewhere in the developed Countries, but in Ghana, pesticide effects have not been given much attention and research. There is scanty information on the toxicity of pesticides to the agroecosystem and it is against this background that this study sought to assess the effects of these pesticides on agroecosystems in Ghana.

The research consisted of questionnaire survey, field work and laboratory experiment using earthworms. Questionnaires were administered to 120 farmers in 120 households at Akumadan in Ashanti region. The field work was also conducted in Akumadan to find out the environmental and health problems which have arisen due to the handling and usage of chemicals. The laboratory experiment used 300 earthworms in groups of 6 and each group consisted of 6 earth pots in which 10 earthworms were introduced. Five of the groups were exposed for 14 days to different concentrations of Lambda Cyhalothrin insecticide commonly used by farmers in Akumadan while the sixth group was set up as control using only tap water for the same period.

The results generated from the questionnaire survey indicated that the use of pesticides could cause health problems to farmers. The field work also indicated that pesticide application could be toxic to non-target organisms. The laboratory experiment also indicated that pesticides could be toxic to the earthworms exposed with the highest mortality of 34% recorded at the 0.64ppm concentration. The calculated LC_{50} value was 0.632ppm.

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GLOSSARY

Adult worms - mean a condition of the worm exhibiting a clitellum in the anterior of the body.

Clitellum - means a glandular portion of the anterior epidermis, appearing as saddle-shaped or annular, usually differentiated externally by color.

LC50 - experimentally derived concentration of test substance that is estimated to kill 50 percent of a test population during continuous exposure over a specified period of time.

Ppm - Parts Per Million

FAO - Food and Agriculture Organization

WHO - World Health Organization

DDT - Dichlorodiphenyltrichloroethane

PPE - Personal Protective Equipment

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Worldwide, agricultural lands comprise 50% of all useable land according to a 2000 FAO assessment on forest resources (FAO, 2001). The same report states that pesticide usage has increased by 854% from 1961 to 1999. These facts highlight the potential problems in conserving biodiversity in agricultural ecosystem where pesticides are used (Reinecke and Reinecke, 2005).

1.1.1 Intensification of Pesticides Use

Humans have utilized pesticides to protect crops and agricultural produce before 2000 BC. The first known pesticide was elemental sulfur dusting used in ancient Sumer about 4,500 years ago in ancient Mesopotamia. By the 15th century, toxic chemicals such as arsenic, mercury and lead were being applied to crops to kill pests. In the 17th century, nicotine sulfate was extracted from tobacco leaves for use as insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum, which is derived from chrysanthemum, and rotenone, which is also derived from the roots of tropical vegetables (Miller, 2002).

In the 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread (Daly et al., 1998).

Until the 1950s, arsenic-based pesticides were dominant (Ritter, 2009). Organochlorines such as DDT dominated and were very effective insecticide, but they were replaced in the U.S. for example by organophosphates and carbamates by 1975 due to its bioaccumulation properties.

Since then, pyrethrin compounds have become the dominant insecticide to control pest (Ritter, 2009).

1.1.2 Benefits of Pesticide Application

During the latter half of the 20th century the development and use of pesticides had increased tremendously and pesticide use has now become an integral component of agricultural farming systems in most developed countries (AATSE report, 2002). It therefore goes without saying that the use of pesticides in modern agriculture to control pests and diseases has significantly increased global food production. Pesticides use has become an integral part of Ghanaian agriculture, being used on cocoa and cotton plantations, vegetable farms, rice and corn fields etc. It is estimated that 87% of farmers in Ghana use pesticides to control pests and diseases on vegetable farms alone (Dinham, 2003).

Usage of pesticide has increased steadily worldwide since the 1960s. It has largely been responsible for the "green revolution", i.e. the massive increase in food production obtained from the same surface of land with the help of mineral fertilizers (nitrogen, phosphorus and potassium), more efficient machinery and intensive irrigation. The use of pesticides has helped to significantly reduce crop losses and improved the yield of crops such as corn, maize, vegetables, potatoes and cotton (Dinham, 2003).

1.1.3 Effects of Pesticide on Environment and Health

Pesticides application is generally expected to have little adverse effect on the environment when used as directed by the manufacturers. Unfortunately, problems are encountered with some pesticides, due to their build-up in the environment and subsequently the food chain. Notwithstanding the beneficial effects of pesticides, their adverse effects on the environment and human health have been well documented worldwide and constitute a major issue that gives rise to concerns at local, regional, national and global scales (Planas *et al*., 1997; Huber *et al*., 2000; Kidd *et al*., 2001; Ntow, 2001; Cerejeira *et al*., 2003).

In the 1960s, it was discovered that DDT was preventing many fish-eating birds from reproducing, which was a serious threat to biodiversity. According to Carson (1962), the best-selling book "Silent Spring" the harmful effects that chemical pesticides had had on the environment magnifies biologically and that many of the long-term effects that these chemicals might have had on the environment, as well as on humans, were still unknown.

The agricultural use of DDT is now banned under the Stockhom Convention on Persistent Organic Pollutants because of their persistence and potential to bioaccumulate. However, it is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes (Lobe, 2006).

Residues of pesticides contaminate soils and water, persist in crops, enter food chains, and finally are ingested by humans with foodstuffs and water. Furthermore, pesticides can be held responsible for contributing to biodiversity losses and deterioration of natural habitats (Sattler *et al.*, 2006).

Pesticides application could alter the structure (species richness, density, and biological diversity) and functional activities of ecosystems. It may also alter the self-sufficient nature of natural ecosystems that include plants, herbivores, parasites, predators, and decomposers. Some pesticides are capable of destroying some species totally or significantly reducing the populations of others. When the diversity of the ecosystem is reduced sufficiently, then food chains may be shortened or altered in diverse ways (SCOPE 49, Accessed March 2, 2011).

There have been reported instances of pest resurgence, development of resistance to pesticides, secondary pest outbreaks and destruction of non target species. High pre and post harvest losses due to pests are a major problem for the productivity in the agricultural sector (Ansong *et al*, 2009).

It has been established that pesticides could become a nuisance if they are misused or misapplied. Some of the negative effects include low crop yield, destruction of soil micro-fauna and flora, and undesirable residue accumulation in food crops (Edwards, 1986; Glover-Amengor and Tetteh, 2008).

1.1.4 Effects of Pesticide on Soil and Water

When pesticides are applied to protect crops from pests and diseases, only about 15% of the preparation hits the target. The rest are distributed in the soil and air. (Leonila, 2002). However, the bulk of pesticide residues in soil are generally confined to the upper 5cm of the topsoil (FAO, 2000).

When arable land is sprayed with pesticides to control pests, droplets of the pesticides are inevitably deposited on nontarget areas (Muir *et al*., 2004). Untimely rainfall after spraying events cause runoff and leaching of these pesticides into soil, underground water, and nearby surface waters. Pesticides in soil can move from the surface when they percolate down through the soil. It is therefore possible that the chemicals will impact not only on target species but also on non target organisms in and adjacent to the target areas (Schulz *et al*., 2001).

Characteristics that may influence the leaching of pesticides into soils include the amount of rainfall, soil drainage, mobility of the pesticide and its degradation process, as well as agronomic factors such as timing, rate and method of the pesticide application (FAO, 2000, Wyman *et al*. 1985, Helling and Gish, 1986).

1.2 STATEMENT OF THE PROBLEM

Some of the factors which are thought to have contributed to pesticide poisoning include lack of education among users and handlers, safety precautions as well as poor attitudes to minimize or prevent environmental and health effects (Clarke *et al*, 1997). Available literature supports the fact that a lot of research work has been done on the toxicity of pesticide in agro ecosystem elsewhere in developed countries such as Europe, United States of America and some parts of Asia since the beginning of the 'green revolution', but in Ghana, pesticide effects have not been given much attention and research. There is scanty or not much literature on the effects of pesticide handling and usage. It is against this background that this study sought to assess the effects of these pesticides on agro ecosystem in Ghana.

1.3 JUSTIFICATION OF THE STUDY

In advanced countries, strict pesticide regulation and enforcement mechanisms are put in place to ensure their safe use and proper handling. The control measures put in place further ensure that approval for the sale and use of pesticide is based on scientific data that support its effectiveness against target pests and that it is not unduly hazardous to beneficial organisms.

In most developing countries however, pesticide use is based solely on manufacturer's recommendations. These recommendations include data on toxicological and environmental properties of the pesticides, though useful, may not be appropriate under local conditions since they were tested under different agro-climatic and socio-cultural conditions.

In Ghana, pesticides use continues to increase as agricultural production intensifies. However, associated with the increased use of pesticides are environmental and health problems which have arisen due to indiscriminate use and inappropriate handling of the chemicals. Recommended practices are not sufficiently adhered to as the local farmers/applicators rely mainly on their personal experiences, unsubstantiated claims by neighbors and general poor attitudes due to some measures of illiteracy and ignorance.

This study therefore sought to assess the effects of pesticides on the health of human beings and non-target organisms with particular reference to earthworms. The study also sought to assess the effect of the use of such chemicals to control pests in Ghana.

1.4 AIM OF THE STUDY

The aim of the study was to determine the effects of pesticides application on agro ecosystem and non target organisms

The specific objectives of the research included the following:

1. The most frequently used pesticides by the farmers.
2. The knowledge of farmers regarding the use of pesticides, health consequences and means of preventing pesticide poisoning.
3. The work practices that increase or minimize exposure to some pesticides
4. The attitudes underlying failure to strictly observe manufacturers instructions and other safety practices and precautionary measures.

The use of many insecticides which are effective against crop pests is not always accompanied by a significant increase in the yield of crops over a long period. This might be due to several factors including toxic effects of pesticides to beneficial insects.

CHAPTER TWO

2.0 LITERATURE REVIEW

Agro ecosystem is plagued with a number of hazards resulting from the rapid decline in quality of pesticides and their effective regulation and control at retail level following privatization and trade liberalization in most developing countries. Most of these pesticides are classified as dangerous because they contain potentially hazardous ingredients (FAO, 2002).

Exposure to these hazardous ingredients could affect agro ecosystems by disrupting natural equilibriums, these effects can be observed by measuring the stability of populations, nutrient cycling, species diversity, interspecies food chains, primary production and energy flow through trophic levels and pollination. (SCOPE 49, accessed March 2, 2011)

The behavior of pesticides in agro ecosystem depends on stability, physico chemical properties, the nature of the medium into which pesticides are applied, the organisms present in the ecosystem, and the prevailing climatic conditions (Graham-Bryce, 1981).

2.1 PESTICIDE

Pesticide can be defined in several ways according to its functions. It can therefore be defined as a chemical substance, biological agent (virus or bacterium), antimicrobial, disinfectant or device used against any pest including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of foodstuff, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term pesticide also includes substances intended for use as plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAO, 2002).

Pesticides are also toxic chemicals that are both ubiquitous and unique. Unlike other toxic chemicals, they are designed to kill, repel, or otherwise harm living organisms (U.S. Environmental Protection Agency (EPA) 2005), and they are one of the few toxic substances that are intentionally applied to the environment (National Research Council, 1993).

Pesticides encompass a great range of diverse substances, falling into several broad groupings such as herbicides, insecticides, fungicides, molluscicides, nematicide etc. according to the target organism (Gevao and Jones 2002).

2.2 PESTICIDE FORMULATIONS

The active ingredients in a pesticide product are the chemicals that control the target pest. A typical pesticide product also has other ingredients called 'inert' (or inactive) ingredients.

In ordinary usage, the word 'inert' refers to something that is physically, chemically, or biologically inactive believing to mean harmless ingredients (EPA 1997). In fact, an inert ingredient may have biological activity of its own, it may be toxic to humans, and it may also be chemically active (USEPA, 2002).

These inactive ingredients are added to dilute the pesticide or render it safer, more efficient, easier to measure, mix, and apply, and more convenient to handle. The process of preparing mixtures of active ingredients with other materials is known as formulation. Pesticides are formulated to increase their efficiency, make them easy to handle and less hazardous to people and the environment.

2.3 ROUTES OF EXPOSURE

2.3.1 Inhalation

Certain pesticides may be inhaled in sufficient amounts to cause serious damage to nose, throat and lung tissues, or to be absorbed through the lungs into the bloodstream. Vapors and very small particles pose the most serious risks having the highest hazard with respect to worker exposure. The hazard of poisoning from respiratory exposure is great because of the rapid and complete absorption of pesticides through lung tissues. Working with wettable powders can be more hazardous because the powder may be inhaled during mixing operations and usually contain concentrated pesticide active ingredient (Adams, 1995).

Many pesticides that produce vapours provide a warning of their presence by their smell or by causing irritation of the eyes, nose and throat. However, some pesticide vapours have little smell and provide little warning of their presence (Adams, 1995).

2.3.2 Ingestion

Farmers have mistakenly drunk from bottles containing pesticides or have been poisoned by drinking water stored in pesticide contaminated containers. Farmers handling pesticides or application equipment can also consume excessive levels of pesticides if they do not wash their hands before eating or smoking (Adams, 1995)

2.3.3 Oral or Mouth & Gastrointestinal tract

The most severe poisoning usually result when pesticides are taken in through the mouth. Pesticides can be ingested by accident, through carelessness or intentionally. The most frequent cases of accidental oral exposure are those in which pesticides have been taken from their original labeled container and put into an unlabelled bottle or food container (Adams, 1995)

2.3.4 Skin or Dermal Exposure

In work situations, skin absorption is the most common route of poisoning from pesticides. Absorption will continue as long as the pesticide remains in contact with the skin. The rate at which dermal absorption occurs is different for each part of the body. The head especially the scalp, ear canal, armpits and the genital areas are particularly vulnerable. This absorption may occur as a result of a splash, spill or drift when mixing, loading or applying the pesticide. It may also result from exposure to residue on application equipment, protective clothing or treated surfaces after pesticide application (Adams, 1995)

The hazard from skin absorption increases when workers are mixing pesticides because they are handling concentrated pesticides that contain a high percentage of active ingredients without personal protective equipment.

2.4 PESTICIDE TOXICITY

Although there are benefits to the use of pesticides, there are also drawbacks such as potential toxicity to humans and other animals.

2.4.1 Acute toxicity

Acute toxicity refers to effects of a single exposure or repeated exposure to pesticides over a short period of time. Immediate health effects include irritation of the nose, throat, and skin causing burning, stinging and itching as well as rashes and blisters. Nausea, dizziness and diarrhea, unconsciousness, nasal and oral secretions, vomiting, headaches, abdominal pain, skin and eye problems coma or even death are also common (Ecobichon, 1996).

In many cases, symptoms of pesticide poisoning mimic symptoms of cold or flu. Since pesticide related illnesses appear similar or identical to other illnesses, pesticide poisonings are often misdiagnosed and under reported. Immediate symptoms may not be severe enough to prompt an individual to seek medical attention, or a doctor might not even think to ask about pesticide exposure.

Acute poisoning of agro pesticides is a well established public health problem across the developing world with an estimated 300,000 deaths globally every year (Gunnell and Eddleston, 2003). The WHO estimates that pesticide ingestion is the most common method of suicide worldwide and has thus launched a Global Pesticides and Health Initiative aimed at developing strategies to reduce the health impact of pesticides (Bertolot *et al*. 2006; WHO, 2006)

Pesticides also contaminate drinking water and food crops, and high-dosage pesticide use in the production of fruits and vegetables can cause serious health hazards to consumers (Pimentel *et al*., 1992).

Poisoning of farmers due to field exposure to pesticides occurs frequently, especially in developing countries (Sivayoganathan *et al*., 2000).

2.4.2 Chronic toxicity

Chronic toxicity also refers to the effects of pesticide over a long period of time or repeated lower levels of exposure to pesticides. Chronic health effects include cancer and tumors, brain and nervous system damage, still birth, sterility, spontaneous abortion, infertility and other reproductive problems, damage to the liver, kidneys, lungs and other body organs. (Ecobichon, 1996). DDT for instance caused eggshell thinning in several high trophic level avian species and sufficient impact on reproduction to result in population declines (Risebrough, 1986). Likewise, the effects on fish occurred largely during the reproductive cycle at the time that the yolk sac was absorbed (Burdick *et al*., 1964).

Pesticide exposure is also associated with chronic health problems such as respiratory, memory disorders, dermatologic conditions, cancers, depression, neurologic deficits, miscarriages, and birth defects (Arcury *et al*., 2003; Strong *et al*., 2004).

Chronic effects may not appear for weeks, months or even years after exposure, making it difficult to link health impacts to pesticides. Pesticides have been implicated in leukemia, lymphoma and cancers of the brain, breasts, prostate, testis and ovaries problems.

2.5 CLASSES OF PESTICIDES

According to the Council on Scientific Affairs, American Medical Association (1997) pesticides can be classified by target organism, chemical structure, and physical state. It can also be classed as inorganic, synthetic, or biological (biopesticides). Many pesticides can be grouped into chemical families prominent among them include organochlorines, organophosphates, pyrethroids and carbamates. Pesticides can also be classified based on target pest (insecticides, herbicides, fungicides, etc.), chemical nature (organic, inorganic and synthetic forms), mode of action (contact, systemic, etc.) and their use (selectivity, foliage, etc.). Other Classifications are based on formulation characteristics such as dry (dust, granules, etc.), liquid (water soluble, emulsifiable concentrates, ultra low volume, etc.), gaseous (aerosols, fumigants, smoke/fog generators, etc.) and persistence or non persistence. It can also be classified according to WHO/FAO classification (class i, ii, iii, and iv).

2.5.1 Organophosphates & Carbamates

These pesticides are like nerve gas. They attack the brain and nervous system, interfering with nerve signal transmission. Organophosphates (OPs) and carbamates exert their effects on insects and mammals including human beings by inhibiting the enzyme acetylcholinesterase at nerve endings. The character, degree and duration of acute illness produced by cholinesterase inhibiting OPs and carbamates are all directly related to the dose and route of exposure which in turn determine the degree and rate of inhibition and subsequent accumulation of acetylcholine. Symptoms include neuromuscular paralysis and central nervous system dysfunction. Death may result from respiratory failure secondary to pulmonary oedema, bronchoconstriction and respiratory muscular paralysis. Cardiac arrythmias and epileptic seizures may also occur (Clarke *et al*. 1997).

2.5.2 Organochlorines

Many banned pesticides (including DDT) are organochlorines, although several organochlorine pesticides are still in use in Ghana including DDT. Organochlorines are central nervous system stimulants that can cause tremors, hyperexcitability and seizures. Although these pesticides are generally less acutely (immediately) toxic than organophosphates or carbamates, since they persist in the environment and tend to accumulate in tissue as they pass through the food chain, they are extremely hazardous. Organochlorine pesticide residues and breakdown products are found in human breast milk worldwide, and also in soil and plant and animal tissue.

2.5.3 Pyrethroids

Phyrethroids are synthetic version of an extract from chrysanthemum. They are chemically designed to be more toxic with longer breakdown times and are often formulated with the human body's ability to detoxify the pesticide. The insecticidal properties are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids which are strongly lipophylic and rapidly penetrate many insects and paralyze their nervous systems (Reigart *et al*., 1999).

2.6 EFFECTS OF PESTICIDES ON ECOSYSTEMS

Pesticides have been reported to influence populations of organisms and thus change the interactions and stability among species within ecosystems (Müller *et al*., 1981). The best documented cases of such ecological effects are from agro ecosystems. When insecticides were first used on tropical cotton crops, for example, they controlled the two or three major pests of the crop and greatly increased yields. Within few seasons however, the chemicals reduced populations of parasites and predators, and a number of other arthropod species became serious pests (ICAITI, 1977).

2.7 NON-OCCUPATIONAL EXPOSURE TO PESTICIDE

People who work with pesticide products are aware of their exposures and can take precautions to protect themselves when exposed to pesticides in many environments. The Non-Occupational Pesticide Exposure Study (NOPES) in USA (1994) was designed to assess total human exposure to 32 pesticides and their degradation products in the non-occupational environment. Potential routes of exposure assessed were air (personal, indoor, and outdoor), tap water, food, dermal contact, and carpet dust (Whitmore *et al*; 1994).

2.8 CONTROL OF TOXIC EFFECT OF PESTICIDES

The control of toxic effects of pesticide is basically through the use of Integrated Crop Management (ICM) approach. ICM is a procedure which covers Integrated Pest Management, Integrated Weed Management and Integrated Nutrient Management. The widespread application of conventional agricultural technologies such as herbicides, pesticides, fertilizers and tillage has resulted in severe environmental damage in many parts of the world. ICM arose from the recognition of the need for sustainable and profitable agricultural production systems and concerns about environmental stewardship. ICM programs provide integrated plans for management of soil fertility, soil and water resources, pests, and crop production in a way that sustains agricultural profitability and promotes conservation of biological diversity (Kefi, 2001).

Other control measures are enactment of efficient laws and their strict enforcement to ensure that banned and unregistered pesticides are not used. Awareness should also be created regarding the ill-effects of pesticides use and wearing of personal protective equipment as the last resort to minimize the toxic effects of pesticide.

The Environmental Protection Agency of Ghana has formulated biopesticide Registration and Risk Assessment guidelines to facilitate greater use of biologically based safe and management options with the aim of contributing to a reduction in the use of toxic synthetic chemical pesticides (Cherry, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

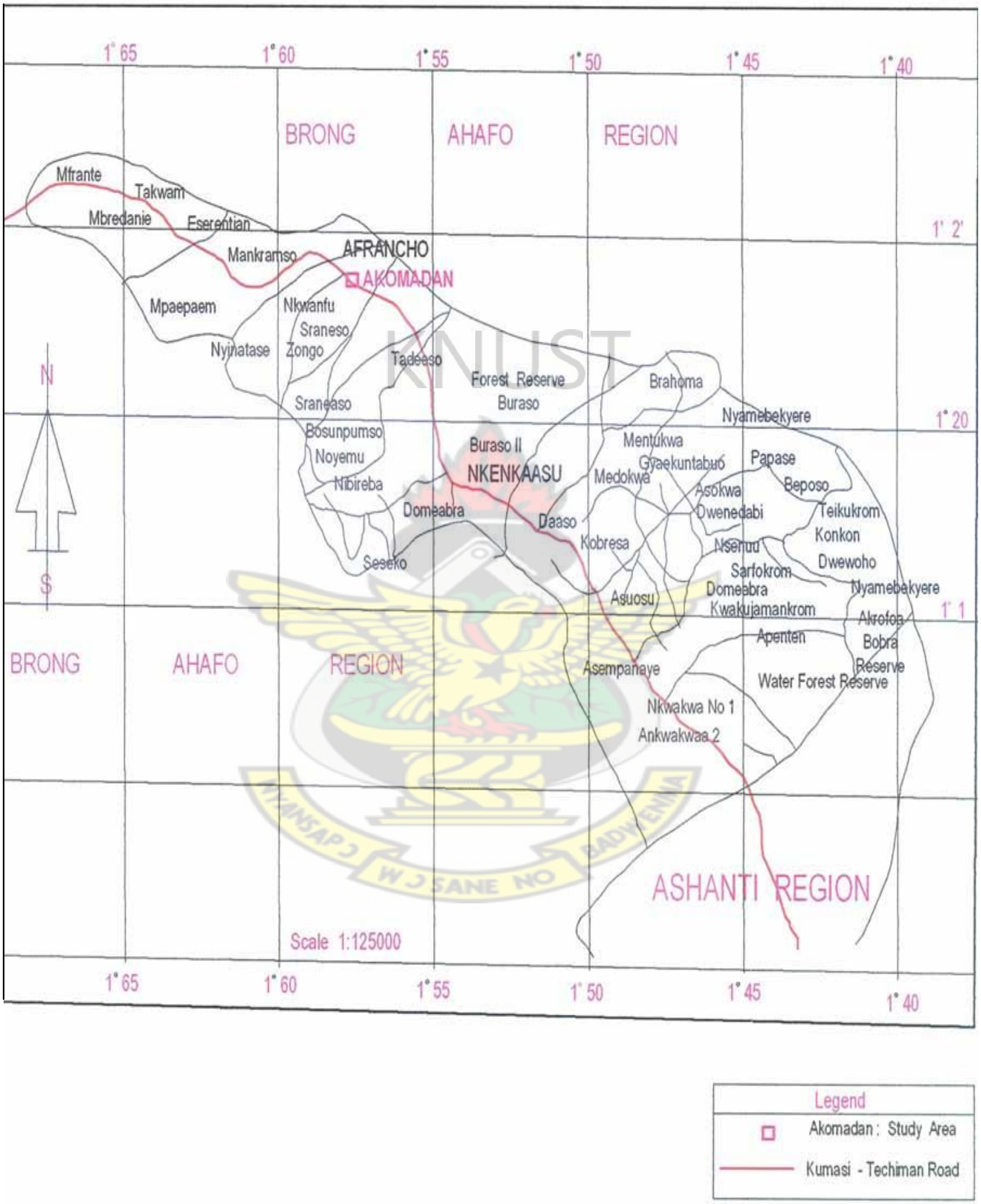
3.1 STUDY AREA

The study was conducted in Akumadan, the capital of the newly curved Offinso North District from the Offinso District.

3.1.1 Location and Boundaries

The area is located in the extreme North-Western part of Ashanti Region of which half of its boundary is bordered by the Brong Ahafo Region in the North and West. It is also bordered on the East by Ejura-Sekyeredumasi District and on the south by Offinso District all in Ashanti Region. According to the Offinso North District Environmental Plan Manual (2008), the district covers a total land area of 741 square kilometers.

Figure 3.1 Map of Offinso North District



3.1.2 Population

The population of Akumadan was estimated in 2008 during the establishment of the Offinso North District at 14,018 with a projection based on an annual growth rate of 5% (National Population Council of Ghana, 2005).

3.1.3 Economic Activities

About 80% of the working population in the district is engaged in agriculture, and an estimated 90% of the workers are in the informal sector of the economy. The rest of the labour force is distributed into commerce (15%), services (3%), and industry (2%). Seventy-three percent (73%) of workers aged 18 years and above are considered self-employed (Botchie, 2000).

3.1.4 Climatic Characteristics

The District experiences semi-equatorial climate with convectional rainfall. Two rainfall seasons are experienced in the district. The major rains start from April to July and the minor from September to mid November. Annual rainfall ranges from 1500mm to 1700mm. Relative humidity is high during the major rainy season reaching its peak of 90% between May and June. Maximum temperature of 30°C is experienced between March and April with mean monthly temperature of about 27°C (Dickson and Benneh, 1988).

3.1.5 Vegetation and Soil Characteristics

The vegetation of the District is moist semi-deciduous forest. The soils are developed from different parent materials. The Kumasi-Offin-Adjamesu series are developed from granite and are deep, well-drained and permeable. They are suitable for the cultivation of food crops such as yam, cassava, maize and vegetables. The Bekwai-Akumadan-Oda compound association is developed from Birimian rock. It is also well-drained and supports the cultivation of food crops and vegetable growing. (Dickson and Benneh, 1988)

Akumadan was chosen as the study area because of the conventional farming activities characterized by regular spraying with pesticides. Pesticide application in these farms is all year round.

3.2. ADMINISTRATION OF QUESTIONNAIRE (SURVEY)

The questionnaire was designed and administered to identify the most commonly used pesticides in the study area. It was also to establish the route of exposure to pesticides, the kind of protective equipment used during application, type of sickness resulting from the pesticides application and the general harmful effects of pesticide on the agro ecosystem.

In all, 120 questionnaires were administered to farmers who basically cultivate tomato. It was a household survey and all the respondents were farmers. Due to the prevalence of illiteracy among the farmers in the area, a face to face interview was scheduled. Only one person was interviewed in a compound house in situations where there were many farmers.

3.3 FIELD WORK

To determine the effects of the pesticides on non-target organisms, 25 x 25 cm quadrats were randomly distributed to a depth of 20cm and 40% formulation of Lambda Super insecticide was applied to pits dug in each of these quadrats.

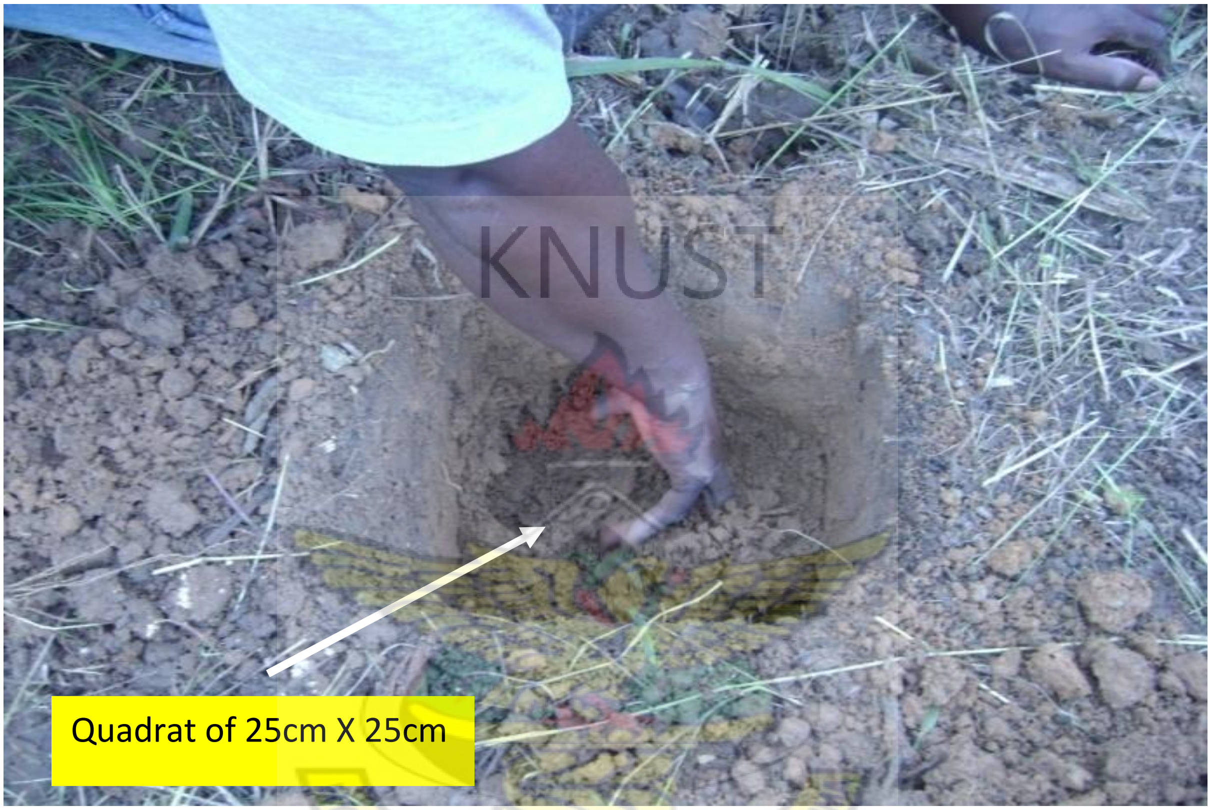


Plate 3.1 Pits of 25 x 25cm to 20cm depth dug in the test tomato farm.

The pits were carefully examined after application of the 40% formulation of the insecticide to pits dug in each of these quadrats and earthworms emerging from these pits were picked up and counted. The worms were identified and their relative abundance determined.

Plate 3.2: Earthworms picked from a Pit, identified and relative abundance determined.

The present work was carried out under field conditions during two successive tomato seasons to determine the toxicity and hazardous effect of pesticides applied to tomatoes on non-target organisms.

Earthworms were collected from a pristine area as control where application of pesticide had not taken place. Their relative abundance was determined. Some of these earthworms were used for the laboratory experiment because they had not much been affected by pesticide.

Plate 3.3 Earthworms Sampled from Pristine Area as Control for the Field Work.

3.3.1 Insecticidal Dilutions

The ratio of 340ml (2 milk tins) Lambda Super insecticide is to 225,000ml (1 barrel) of water as diluent was prepared which was the same formulation used by the farmers on the field. Concentrations were prepared periodically using the same formula and applied to the tomato farm.

3.3.2 Application of Pesticide on Tomato Farms

Knapsack spray cans were used to disperse the insecticide concentration onto the tomato crops until the whole farm was completely sprayed. The process was repeated every two weeks until harvest time.

The mode of application of the pesticides on the tomato farms was based on the practice used by the local farmers.

3.4 LABORATORY EXPERIMENT

3.4.1 Selection of Insecticide

Selection of insecticides for the laboratory experiment was based on the outcome of the questionnaire survey. Lambda Super was identified as the most common insecticide in use having Cyhalothrin as active ingredient.

3.4.2 Preparation of Insecticide for Treatment

The stock solution of Lambda Super was prepared using the method often used in the field by the farmers i.e. 225,000ml (1 barrel) of water as diluents was used for mixing 340ml (2 milk tins) Lambda Super insecticides. Five different concentrations were made. These concentrations were made from 1% stock solution. The concentrations were 0.04ppm, 0.08ppm, 0.16ppm, 0.32ppm and 0.64ppm.

3.4.3 Earthworms for Experiment

The standard protocol employed by this study was based on the guidelines for the ecological effects test in subchronic earthworm toxicity developed by the United States Environmental Protection Agency (USEPA, 1996) through the process of harmonization that blended the testing guidance and requirements of Pollution Prevention and Toxics (PPT) and that of Organization for Economic Cooperation and Development (OECD) (Appendix B

A total of 300 adult earthworms with well developed clitellum were used for the laboratory work sampled from the study area and kept in the laboratory for the experiment. Individual earthworms used for the experiment did not differ tremendously in size. The worms selected were almost of the same size with a relatively homogeneous age structure. The selected worms were acclimatized in the laboratory for two weeks into the artificial soil substrate which was to be used for the test.

The test was carried out under laboratory conditions using pots. The pots were filled with moistened artificial soil substrate which had already been mixed with the different test insecticide concentrations.

A case-control study design was used in which the control was one pot and the experimental groups (cases) were 5 pots arranged in the order of A, B, C, D, E being the test groups and F being the control group.

The first five groups of A, B, C, D, E represented the five concentrations treatment protocols of 0.64ppm, 0.32ppm, 0.16ppm, 0.08ppm, and 0.04ppm exposed to the insecticide concentrations respectively. The last 'F' group represented the control group exposed to only tap water. The experiment was replicated four times using the same case-control study design.

3.4.4 Evaluation of Toxicity of Earthworms Exposed to Different Concentrations of the Pesticide

The toxicity of the earthworms exposed to different concentrations of the chemical was evaluated by contact and oral methods. At each concentration the pots were filled 50g of soil thoroughly mixed with appropriate concentration of the chemical.

Ten earthworms were placed on the surface of the soil and healthy worms burrowed immediately into the substrate and consequently those remained on the surface after 15 minutes were classified as damaged and replaced. The test containers were then covered and placed in the test chamber. The water content of the soil substrate in the test containers were maintained, losses were replenished as necessary with tap water. The same numbers of earthworms were exposed to tap water only as control. Each rate was replicated four times.

The duration of the test was two weeks and mortality was evaluated on the 7^{th} and 14^{th} days. Mortalities were recorded and percentage mortalities corrected into natural mortality using Abbot's formula (1925). The corrected mortalities were then transformed into Probit values using Finney's Table (1952) (Appendix D). The LC_{50} value was computed using Behrines and Karber formula (1965) (Appendix E)

3.4.5 Examination of Earthworms for Toxicological Defects

After the exposure, earthworms were observed for acute toxicity. Observations were made at the 7^{th} and 14^{th} days and recorded accordingly.

3.5 DATA ANALYSIS

The questionnaire and field data were analyzed using STATA version 9 and Analysis of Variance (ANOVA) in STATA version 9 statistical software. Earthworm mortality data from the laboratory studies was also analyzed using ANOVA in STATA version 9 and Excel 2007. No further test was carried out when ANOVA result was found to be significant.

CHAPTER FOUR

4.0 RESULTS

4.1 QUESTIONNAIRE SURVEY

Gender Distribution of Farmers

The responses to the questionnaire show that majority of the respondents were males constituting 74% of the respondents which is indicative of the fact that farming is a male dominated profession in Akumadan from Offinso District.

Figure 4.1 Gender Distribution of Farmers interviewed

Age

Most of the farmers were adults between the ages of 18 and 60 years constituting 88% of the total number of respondents with only 0.8% of farmers below the age of 17 years. The respondents aged 60 and above constituted 11% of the respondents. These results show that children were therefore not much involved in tomato farming.

Educational Background

According to the survey, tomato farming was the major pre-occupation of most of the farmers. Majority of these farmers had basic education constituting 59% while 21% had secondary education. Those who had no education at all constituted 19% while only 1% had tertiary education.

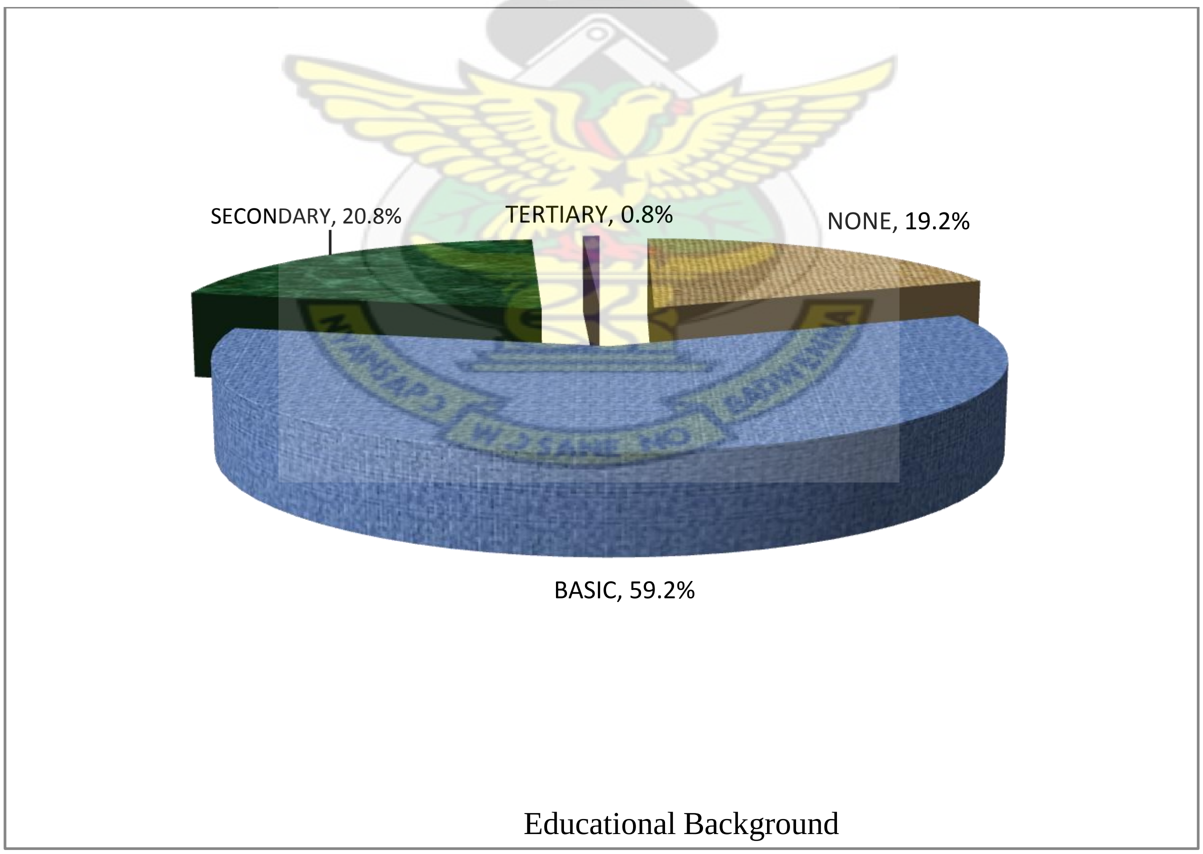


Figure 4.2 Educational Background of Farmers interviewed

Most Common Insecticide used by the tomato Farmers interviewed

The survey revealed that insecticide commonly used by the farmers included kombat representing 24%, kalat representing 19%, controller super represented 22%, lamda super had 24% usage while 10% of the respondents mentioned rocky.

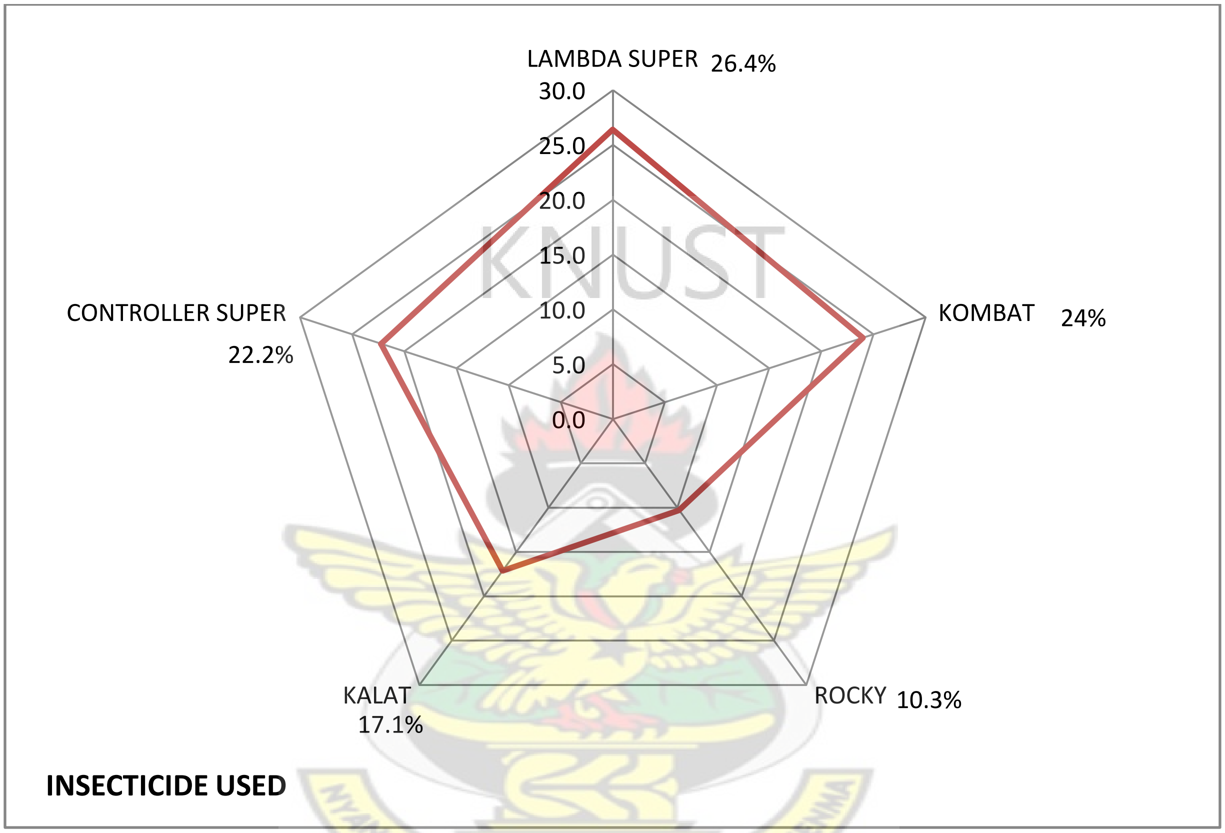


Figure 4.3 Insecticide commonly used by the Farmers from Akumadan

Weedicide Used by the farmers interviewed

In response to the question, most commonly used wedicide was sunphosate constituting 38%. 20% of the respondents preferred using *Condemn* while 21% of them use *Asaasewura*. Those who use *Power* constituted 18% while 2% use *Sarosate*. Those farmers who could not tell the name of the weedicide they normally used constituted 0.2%.

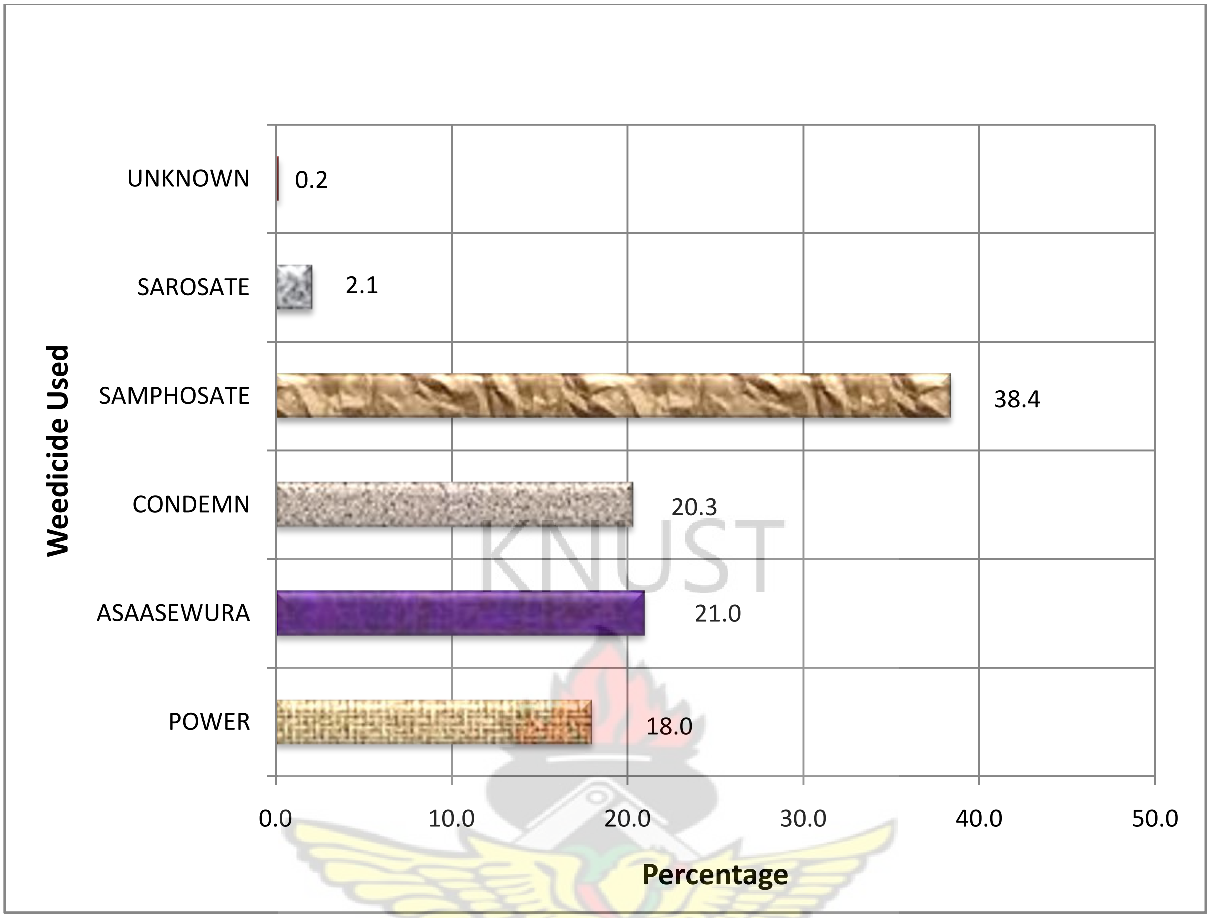


Figure 4.4 Weedicide commonly used by Farmers from Akumadan

Perceived unfavourable weather condition on pesticide applicators

The questionnaire survey revealed that 49% of the farmers believed that rainy days were unfavorable weather condition for the application of pesticide while 39% believed hot days were unfavorable weather for pesticide application (Figure 4.5). Ten percent (10%) of the respondents believed that windy condition was unfavorable weather while 2% believed that weather had no effect on pesticide application and that they would go ahead to apply the chemicals when these conditions were prevailing. The vast majority of farmers (98%) would not apply the chemicals in what they considered unfavorable weather as indicated in figure 4.5.

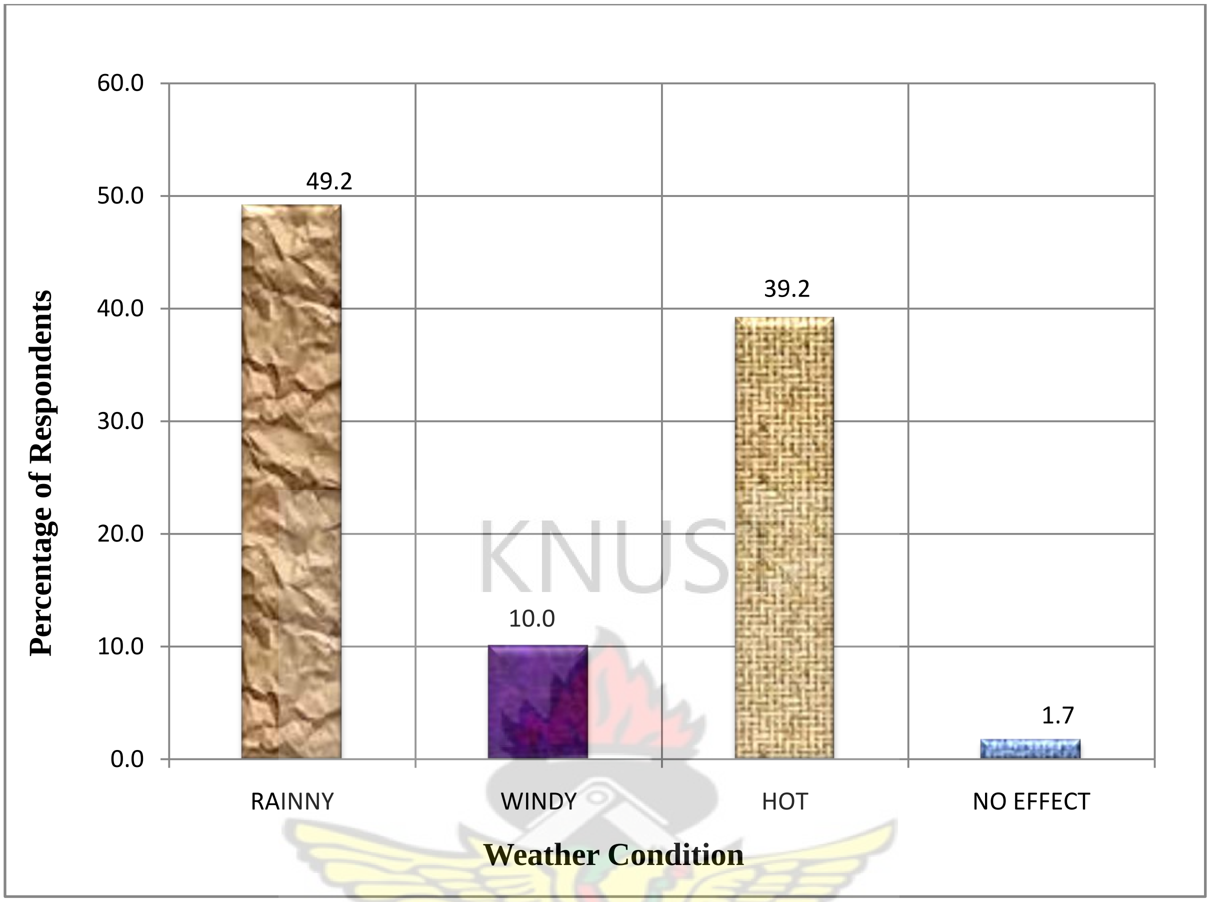


Figure 4.5 Percentage of Farmers indicating of unfavourable weather condition to Pesticide application

Unfavorable and Type of reaction Resulting from Pesticide Application

Among the farmers interviewed, 75.8% of the respondents indicated they have had unfavorable reaction such as body itching, burning skin and blurred vision after pesticide application while 24% had not experienced any reaction at all.

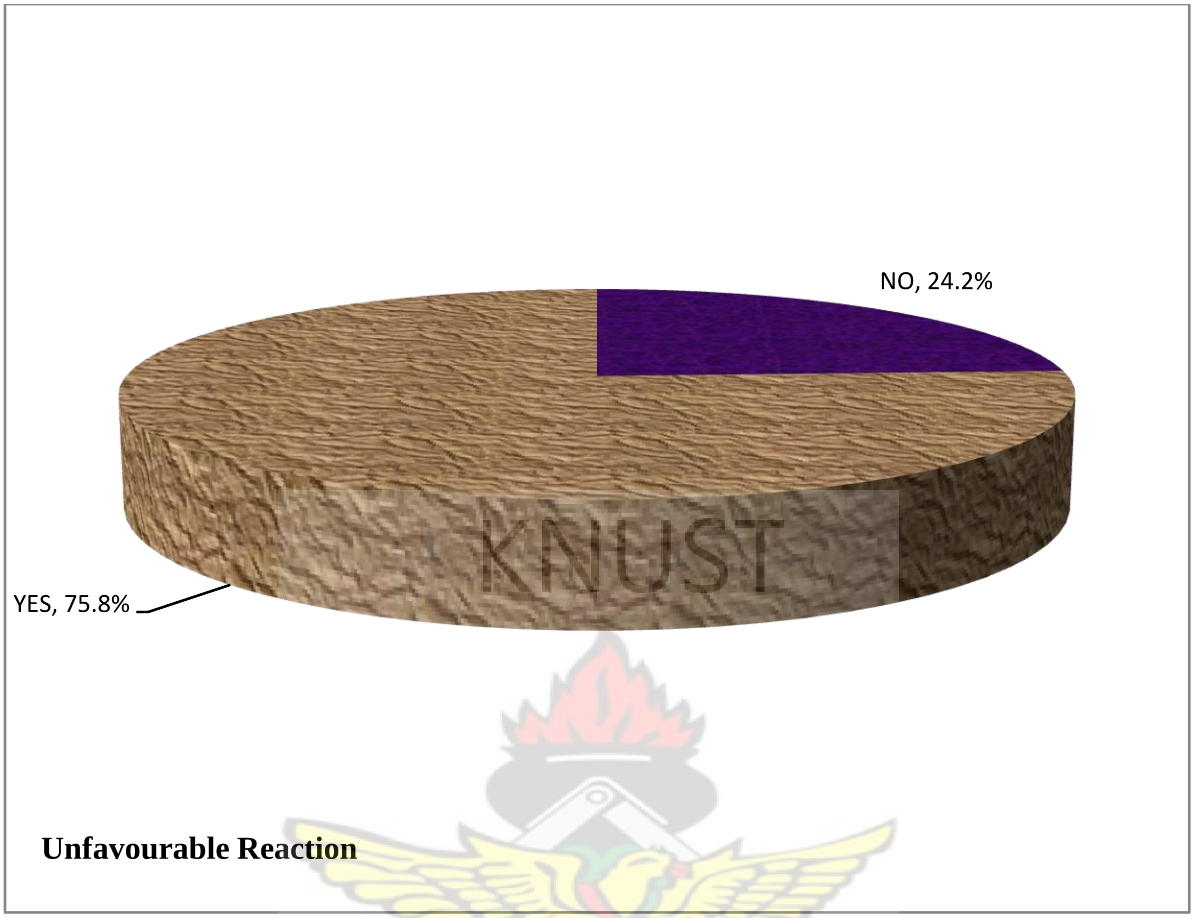


Figure 4.6 Unfavorable Reaction resulting from Pesticide Application

Route of exposure of Pesticide into human body

Among the farmers who have had unfavorable conditions, the major route of exposure of pesticide was through the skin (48%), nostrils (33%), the eyes (13%) while through the mouth accounted for 7%. (Figure 4.7).

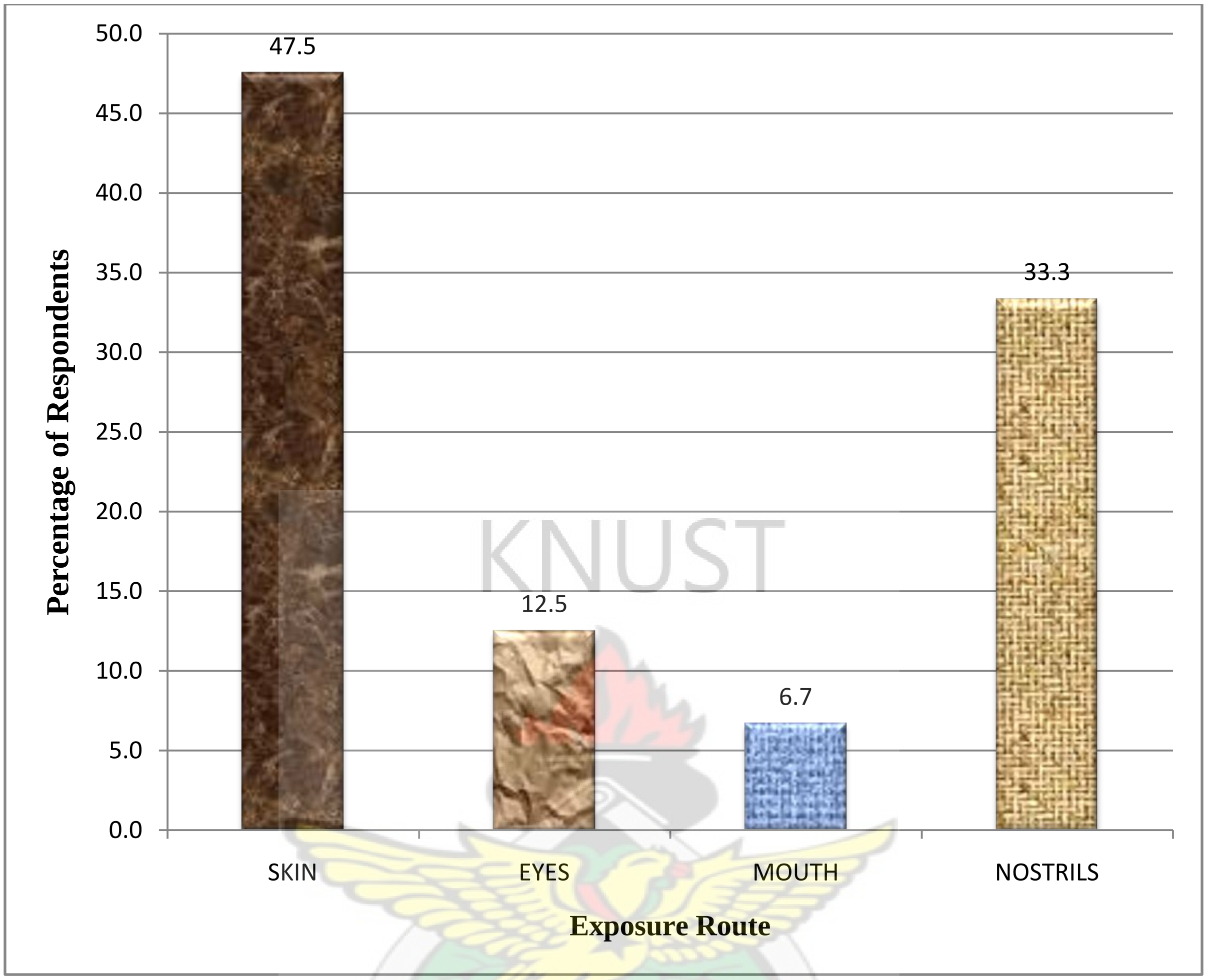


Figure 4.7 Route of Pesticide into human body

Sickness resulting from Pesticide use

When the respondents were asked whether they had ever fallen sick or heard about any sickness resulting from the application of pesticide, 60% responded in the affirmative. When they were asked to identify the sickness type, 46% indicated they had general body weaknes, 17% claimed to have experienced dizziness after mixing of the chemical and after application on the farm, 17% said they had skin disease, 12% attributed feverish condition to pesticide use, 4% had headache after pesticide application, 2% attributed collapsing on the farms to pesticide use, 3% said they normally vomit during pesticide use and only 1% indicated people had stroke resulted from pesticide use.

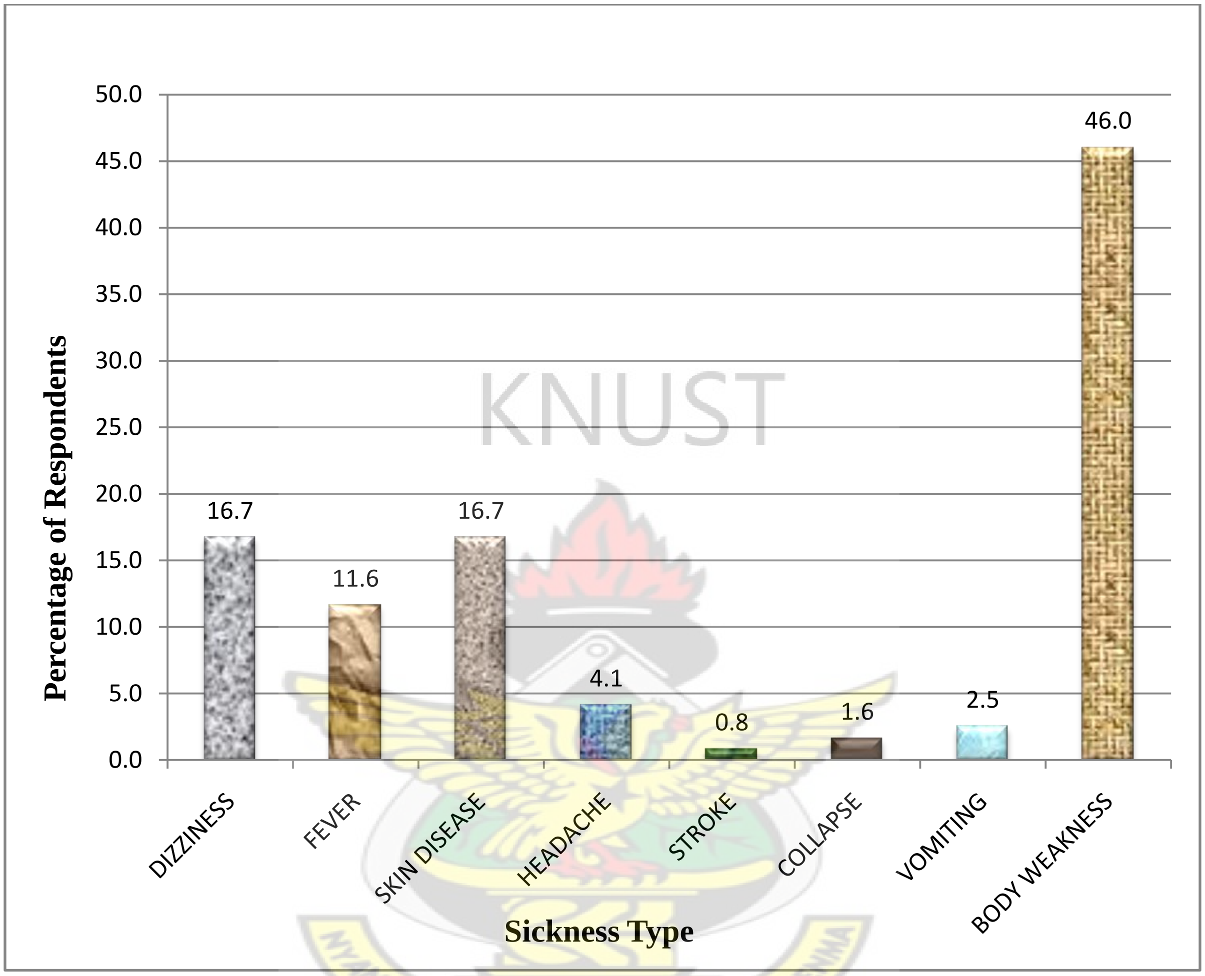


Figure 4.8 Sickness resulting from Pesticide use

Deaths resulting from pesticide poisoning during and after application

The respondents were also asked if they had heard of any human death resulting from pesticide application, 82% answered in the negative. They were also asked whether they have heard of any death resulting from pesticide application, 62% answered in the negative.

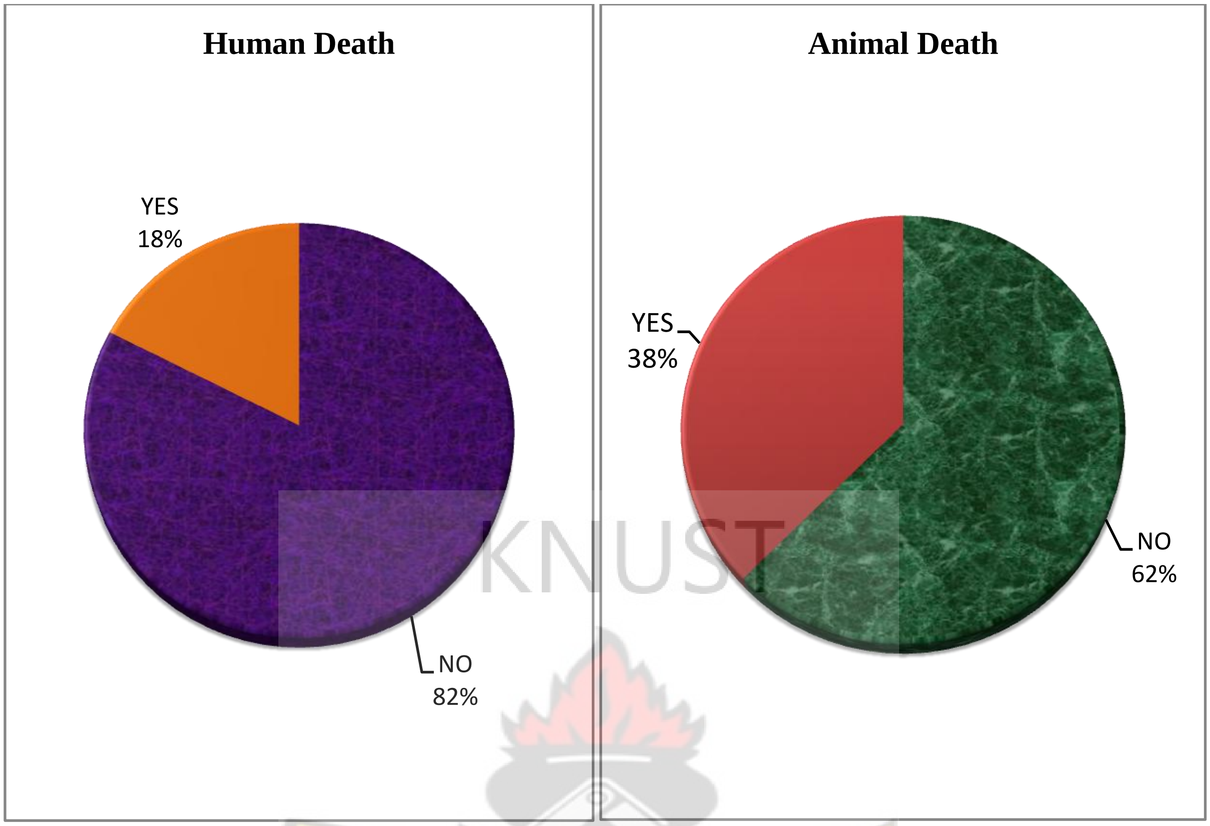


Figure 4.9 Deaths resulting from Pesticide use

Types of Animal killed during pesticide spray

When asked to mention the type of pests and insects killed by the pesticides, the following were mentioned; spiders 16.8%, caterpillars 22.3% and all types of insects 5.8%. Centipedes 5.8%, birds 5%, livestock 3.3%, grasshoppers 19.8%, pets 1.7%, ants 4.9%, earthworms 5%, antelopes 0.8% and 8.8% bees.

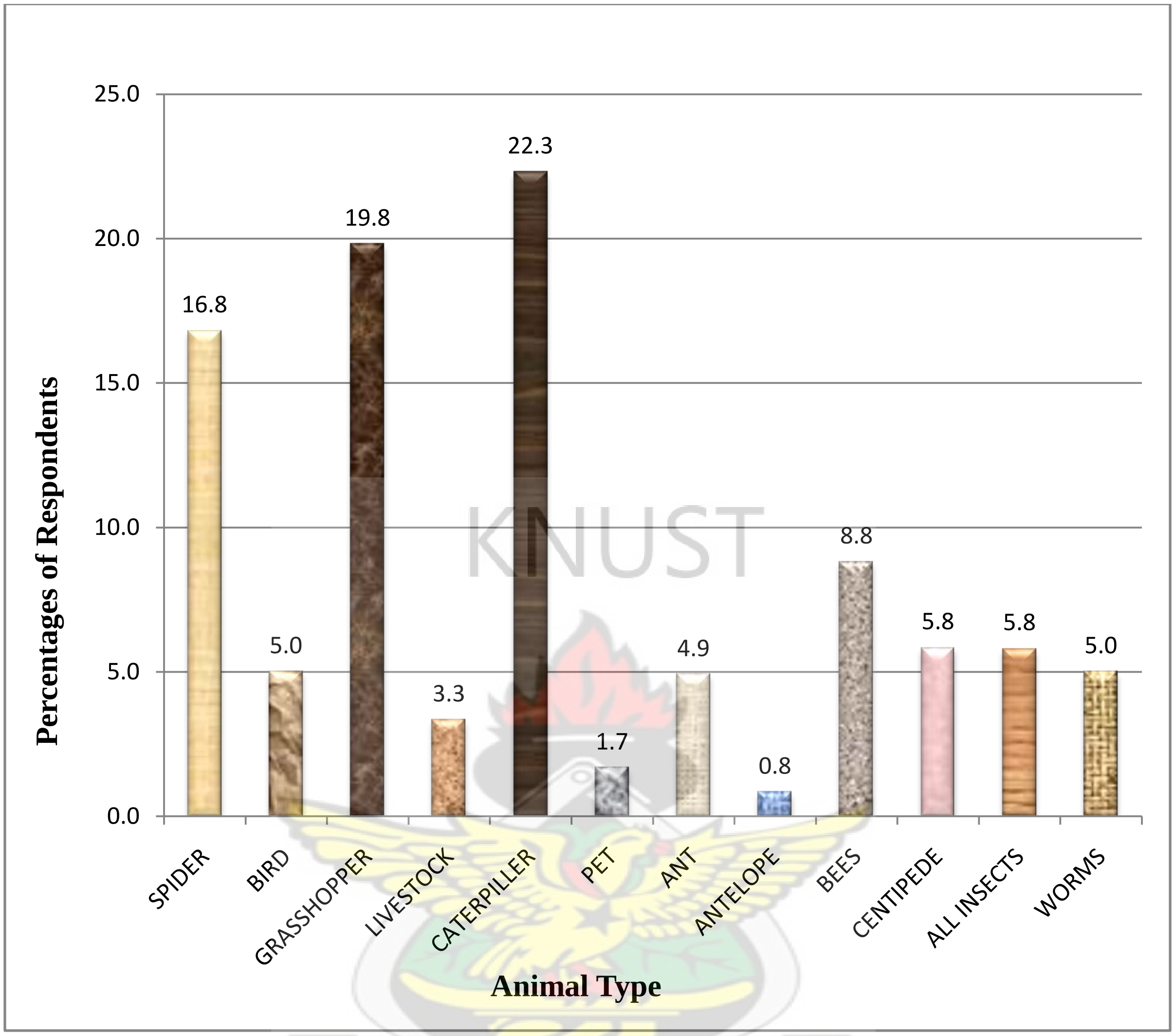


Figure 4.10 Animals killed during Pesticides use

Personal Protective Equipment Used

The use of personal protective equipment is one of the surest ways by which pesticide hazards could be avoided or minimized. The outcome of the survey revealed that a vast majority (58%) did not use personal protective equipment at all, 29% used some form of personal protective equipment but not always, 9% always used the available personal protective equipment while 3% did not know that personal protection equipment was used to minimize or prevent hazards associated with pesticide use.

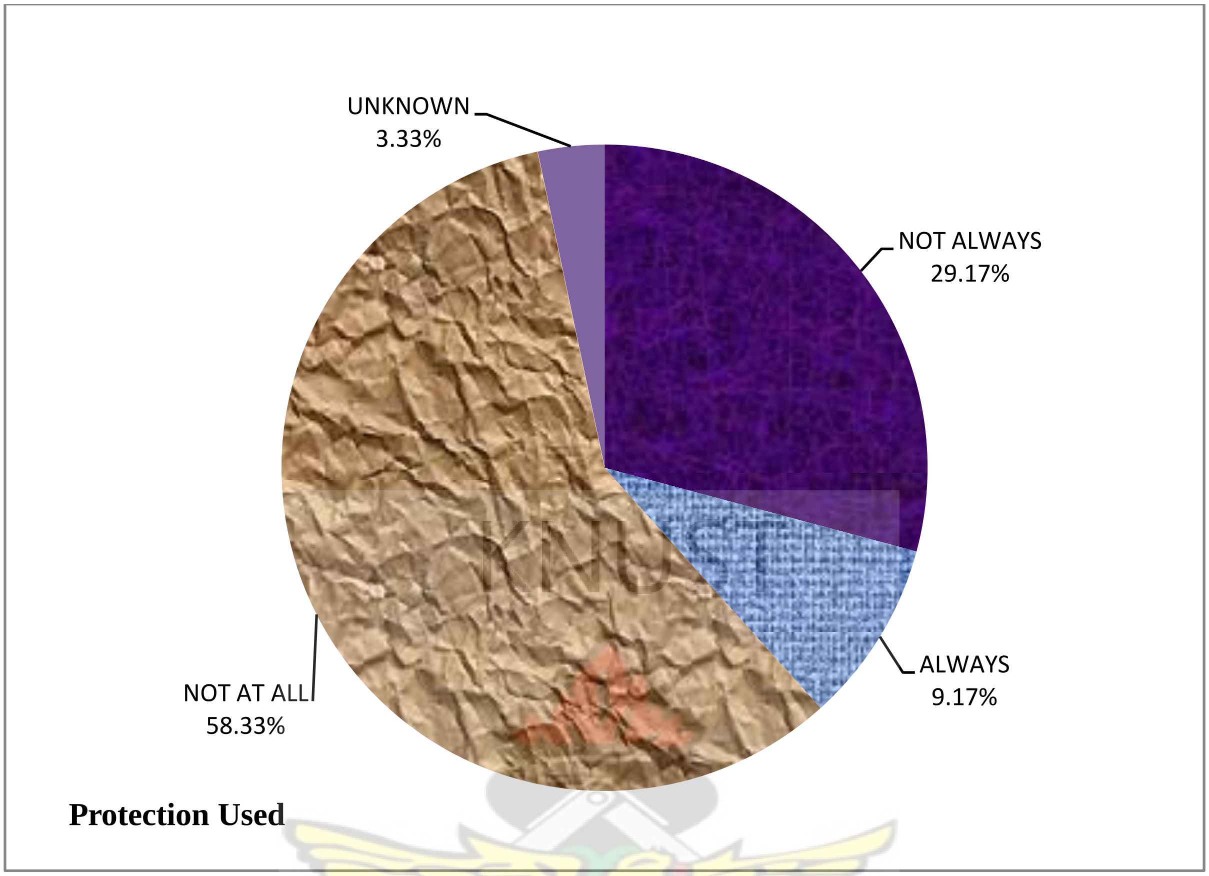


Figure 4.11 Personal Protection Equipment used by Farmers

Among farmers who indicated that they use some type of protective equipment only a minority (2%) used "overalls" protective clothing during pesticide application, 22% used long sleeve shirts, 6% used hand gloves, 4% used respirators, 3% used boots, 16% used shoes, 2% used nose guard and 23% used trousers while only 1% used eye glasses.

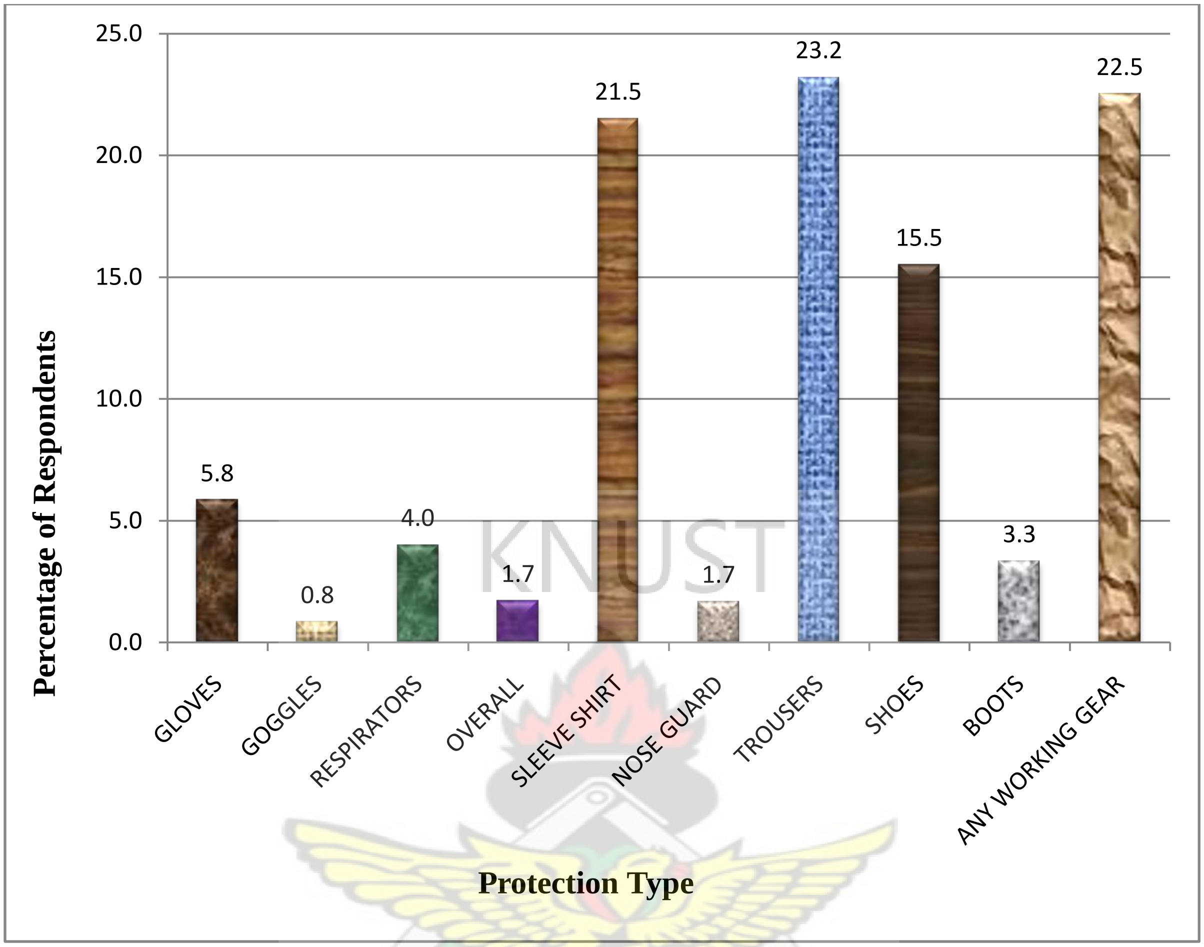


Figure 4.12 Types of Personal Protective Equipment used by the Farmers

Pesticide Containers Disposal

The survey also revealed that 82% of the respondents disposed off pesticide containers any where on the farms after application, 10% burned the containers, 2% buried the containers, 6% hanged the containers on pegs on the farms while only 1% threw into water bodies.

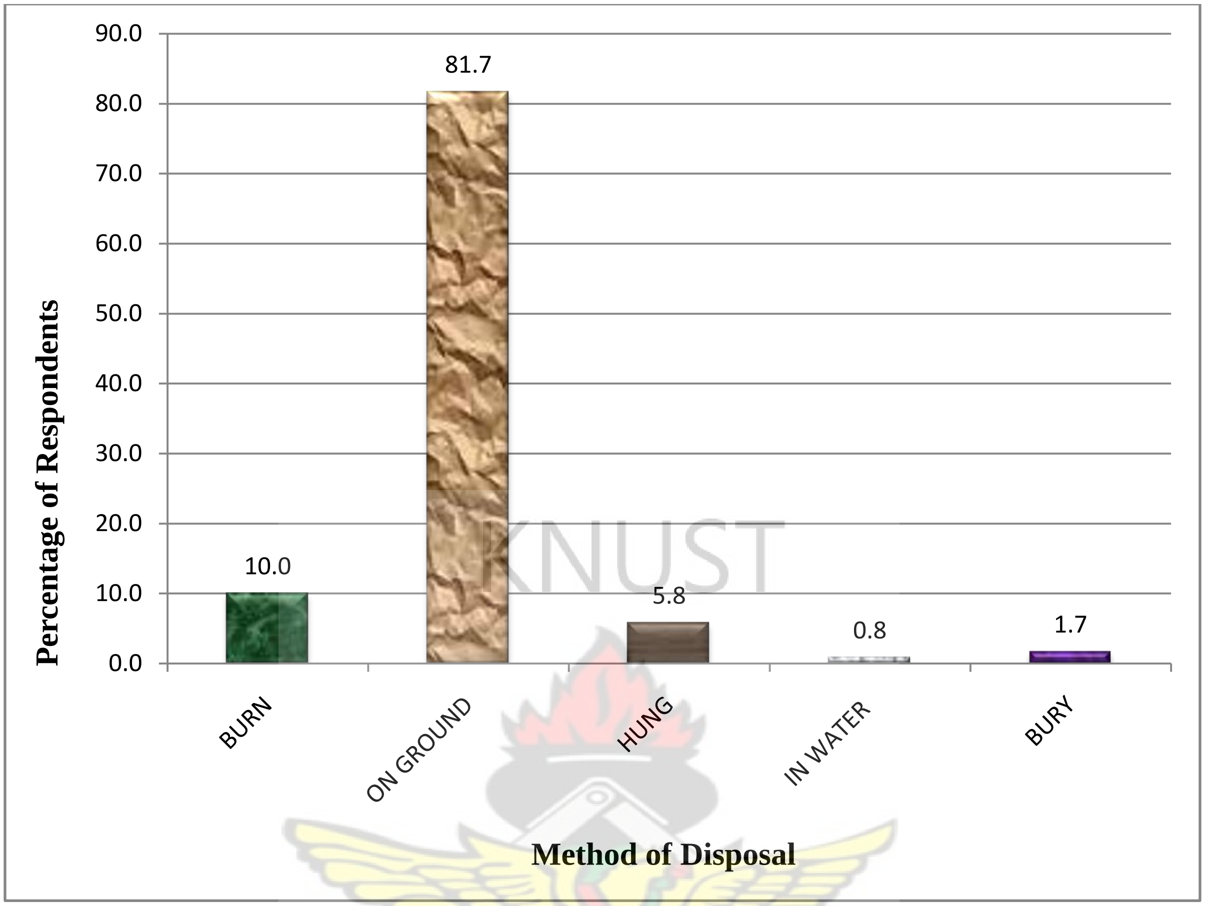


Figure 4.13 Disposal of Pesticide containers

Effects on Non Target Organisms

In response to questions on the possible harmful effects of the pesticides on non target organisms, the respondents indicated that pollinators such as honey bees, spiders and worms were killed. 12% of the respondents revealed that bees were also killed especially when they were sucking the nectar, 8% attributed the death of earthworms to pesticide application, 16% attributed demise of flies to pesticide use, 19% attributed ant's death to pesticide use, and only 1% said frogs died due to pesticide use while 45% attributed spiders' death to pesticide application.

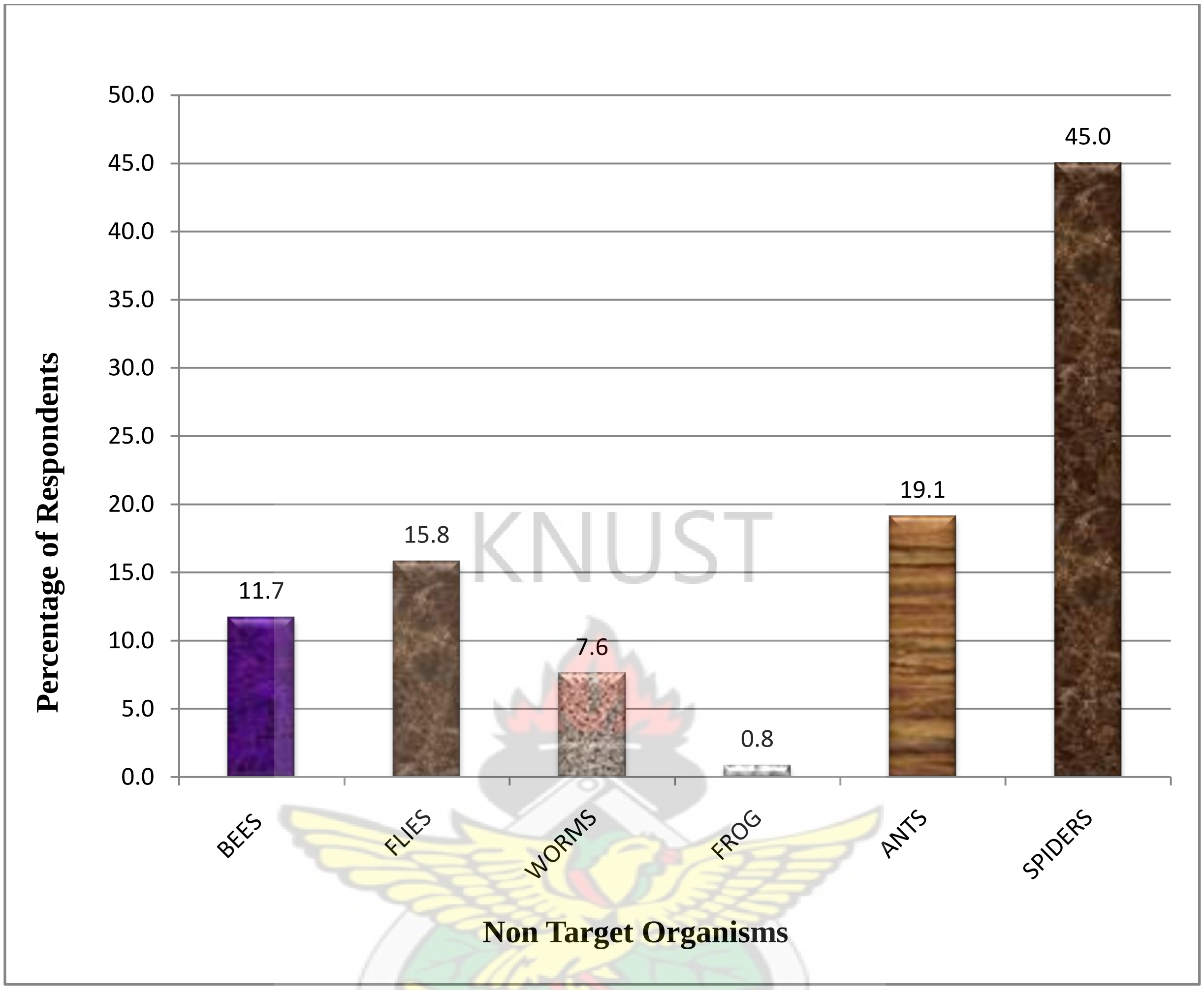


Figure 4.14 Effect of Pesticide on non target organisms

4.2 FIELD WORK/STUDY

4.2.1 EVALUATION OF THE EFFECTS OF THE PESTICIDES ON NON-TARGET ORGANISMS

The effect of the pesticides on nontarget organisms was evaluated using earthworms. The result (Table 4.1) indicated that the chemical (Lambda Super) significantly affected earthworms ($P<0.050$). Detailed analysis of the results also revealed a direct relationship between pesticide concentration and the level of mortality. Expectedly, there was also a correlation between mortality and level of exposure to the chemical (Figures 4.15).

Table 4.1: Mean mortality of earthworms observed after exposure to pesticide

Days After Exposure	No. of Worms Observed	Mean ± SE	Std Dev	95% Confidence Interval
0	85	8.5 ± 0.76	2.51	7.01 to 9.99
7	25	2.5 ± 0.40	1.27	1.7 to 3.3
21	34	3.4 ± 0.40	1.26	2.6 to 4.18
42	24	2.4 ± 0.53	0.84	1.87 to 2.93
63	15	1.9 ± 0.34	1.08	1.2 to 2.6
84	8	0.8 ± 0.29	0.92	0.2 to 1.4

The data (Figure 4.15) represents a summary of days after exposure and number of earthworms observed. The vertical bars represent standard error at each side of the mean.

4.2.2 Statistical Analysis of Variance of Field Data

Table 4.2 Summary Statistics of Analysis of Variance.

Source	SS	Df	MS	F	Prob >F
Between groups	379.08	5	75.82	37.25	0.00
Within groups	109.90	54	2.04		
Total	488.98	59	8.29		

Bartlett's test for equal variances: χ^2 (5) = 15.83 prob > χ^2 = 0.007

The F (5, 54) = 37.25 with P < 0.001; where 5 and 54 are the degree of freedoms for the between groups and within groups respectively as illustrated in Table 4.2 in which the p value (P<0.001), is small indicating that the earthworm counts from the different quadrats were not the same. Hence, the differences in the earthworm population in the various quadrats resulted from the increased effects of pesticide application.

The Bartlett's equal variance χ^2 test (5 d.f) = 15.83 with P = 0.007) however indicated that the equal variance assumption under the ANOVA model was not satisfied. Nevertheless, this does not obliterate the significant difference between the control and the test means as shown in Table 4.2.

4.3 Laboratory Experiment

4.3.1 The Contact Toxicity of Pesticides on Earthworms.

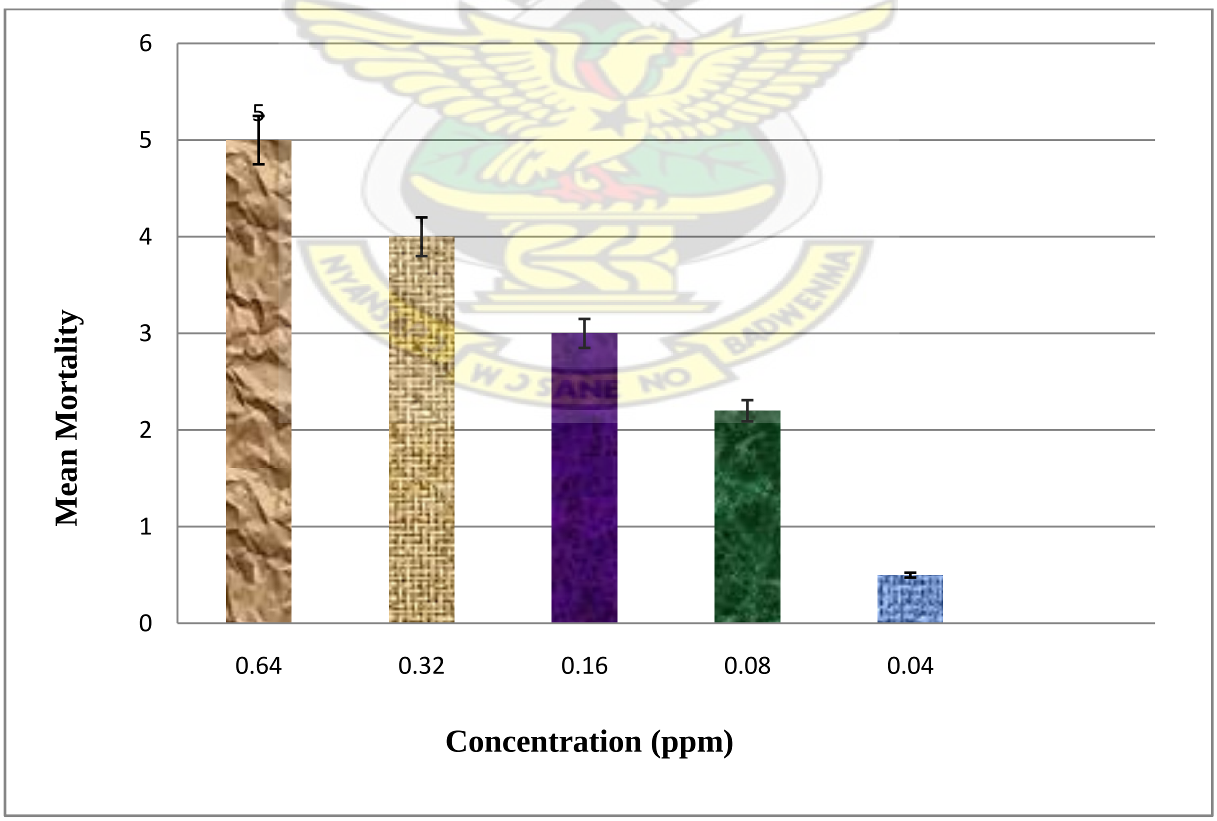


Figure 4.15: Toxicity of Insecticide to Earthworms

Figure 4.15 represents a summary of the various concentrations (ppm) of the chemical and mean mortality of earthworms. Mortality was expressed as means of total number of earthworms counted as dead at each concentration range.

Earthworm mortality was highest at the highest chemical concentration level of 0.64ppm and lowest at the least chemical concentration of 0.04ppm.

There was significant difference of earthworm mortality ($P<0.05$) between the control group (where there was no chemical) and the experimental groups ranging from chemical concentrations of 0.04 to 0.64ppm. Apart from the mean worm mortality at chemical concentration of 0.08 and 0.04ppm where mean mortality was significantly different ($P = 0.003$), there were no significant differences of earthworm mortality between two successive concentrations e.g. between 0.64 and 0.32ppm

Recorded mortality (Table 4.4) was as high as 34% for the concentration of 0.64ppm, and as low as 3% for the concentration of 0.04ppm (Table 4.4). No mortality was however recorded in the control group (which was exposed to only water).

Table 4.4 shows the LC_{50} value of 0.632ppm computed using Behrens and Karber (1953) formula. The percentage mortalities were corrected into natural mortality using Abbot's formula (Abbot, 1925):

$$\textbf{Corrected Mortality} = \mathbf{100} \times \frac{\boldsymbol{P-C}}{\boldsymbol{100-C}}$$

Where P = % mortality in treatment and C = % mortality in control

The corresponding probit values were obtained from the Finney's Table (Finney 1952) (Appendix D)

Table 4.3 Log-Probit Table of Earthworms Exposed to Insecticide Concentration for Two (2) Weeks.

CONC. (PPM)	Mean Mortality	Percentage Mortality	Corrected Mortality	Log Concentration	Probit	LC50ppm
Control	0	0	0	0	0	0.632
0.04	0.5	3.4	3	4.6	3.12	
0.08	2.2	14.8	15	4.9	3.96	
0.16	3	20.5	21	5.2	4.19	
0.32	4	27.2	27	5.5	4.39	
0.64	5	34.1	34	5.8	4.59	

Figure 4.16 show direct relationship between concentration and mortality. The Concentration - Mortality graphs show that mortality of earthworms increased with increase in concentration. An increase concentration of 1ppm is associated with an average corrected mortality of 9 as shown in Fig. 4.16. The strength of the regression is measured by the square of the correlation coefficient (R^2). From Figures 4.16 and 4.17, R^2 of 0.90 and 0.98 respectively show a very strong correlation between concentration and mortality as about 90% of the variation in mortality is explained by the regression of concentration on mortality. In a similar manner, the probit-log concentration graph which is a direct transformation of log-percentage mortality graph (Fig. 4.18) shows a similar R^2 of about 0.69. The Log Concentration graph (Fig. 4.18) shows that mortality increased with increasing concentration of the insecticide and exposure duration.

Figure 4.16, 4.17 and 4.18: Diagram of Concentration – Response Relationship

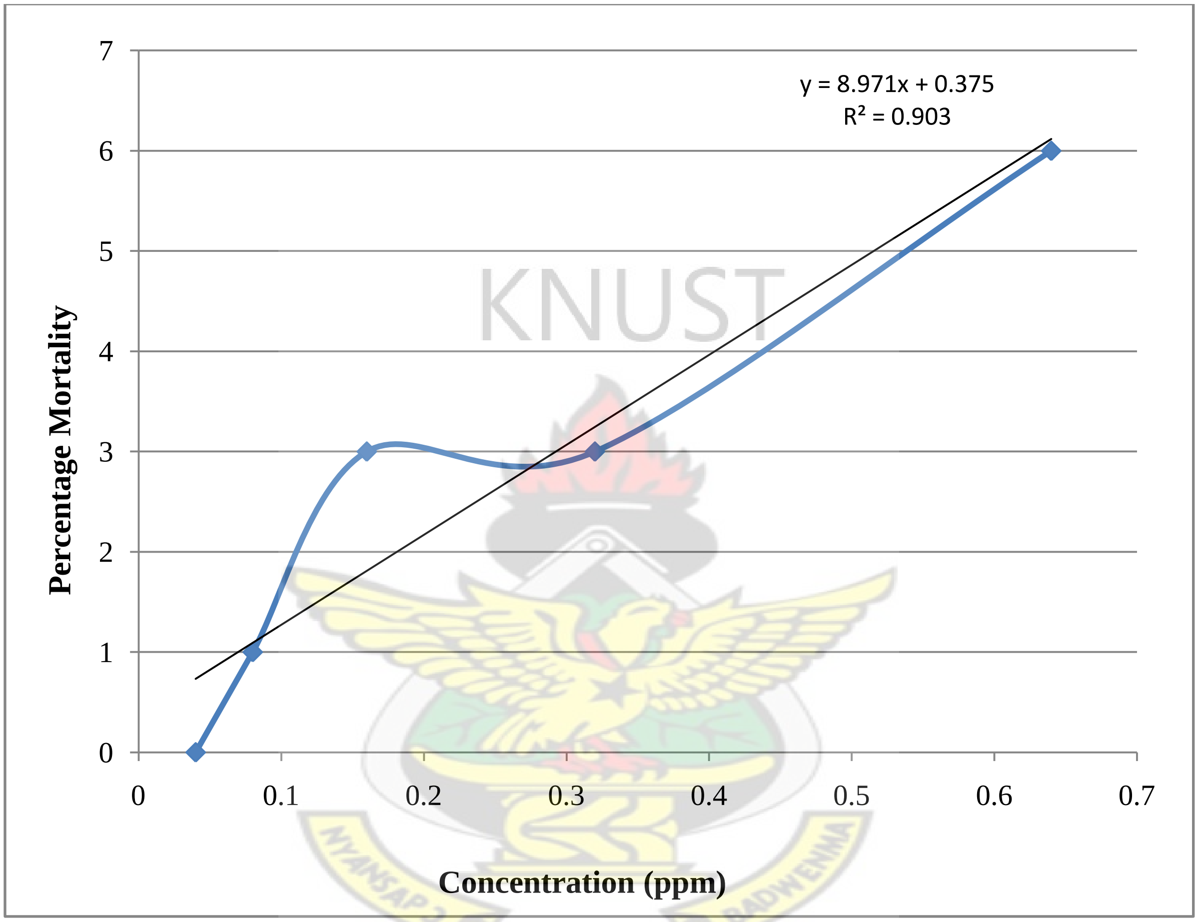


Figure 4.16: Relationship between Concentration and Mortality

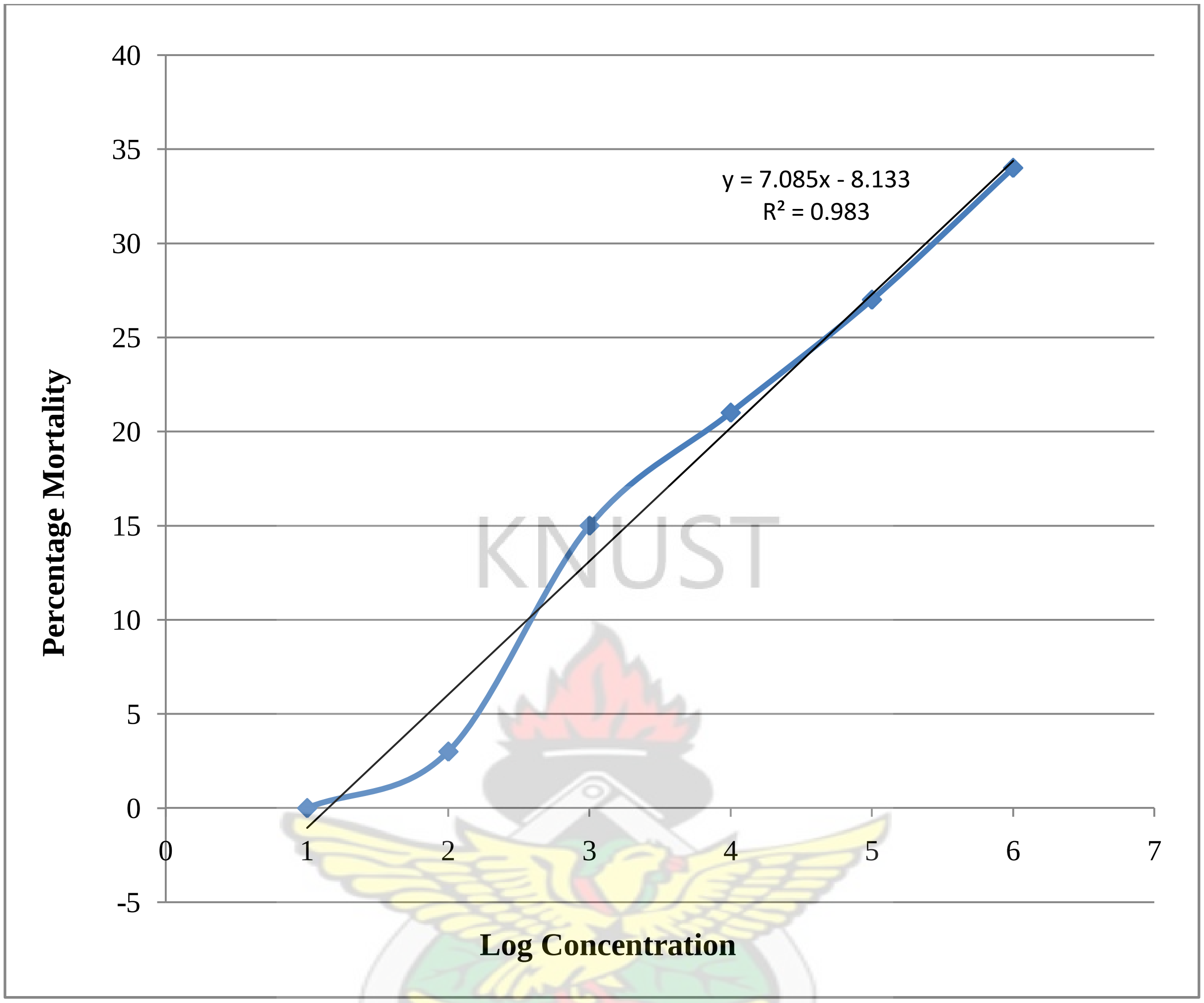


Figure 4.17: Relationship between Log Concentration and Percentage Response

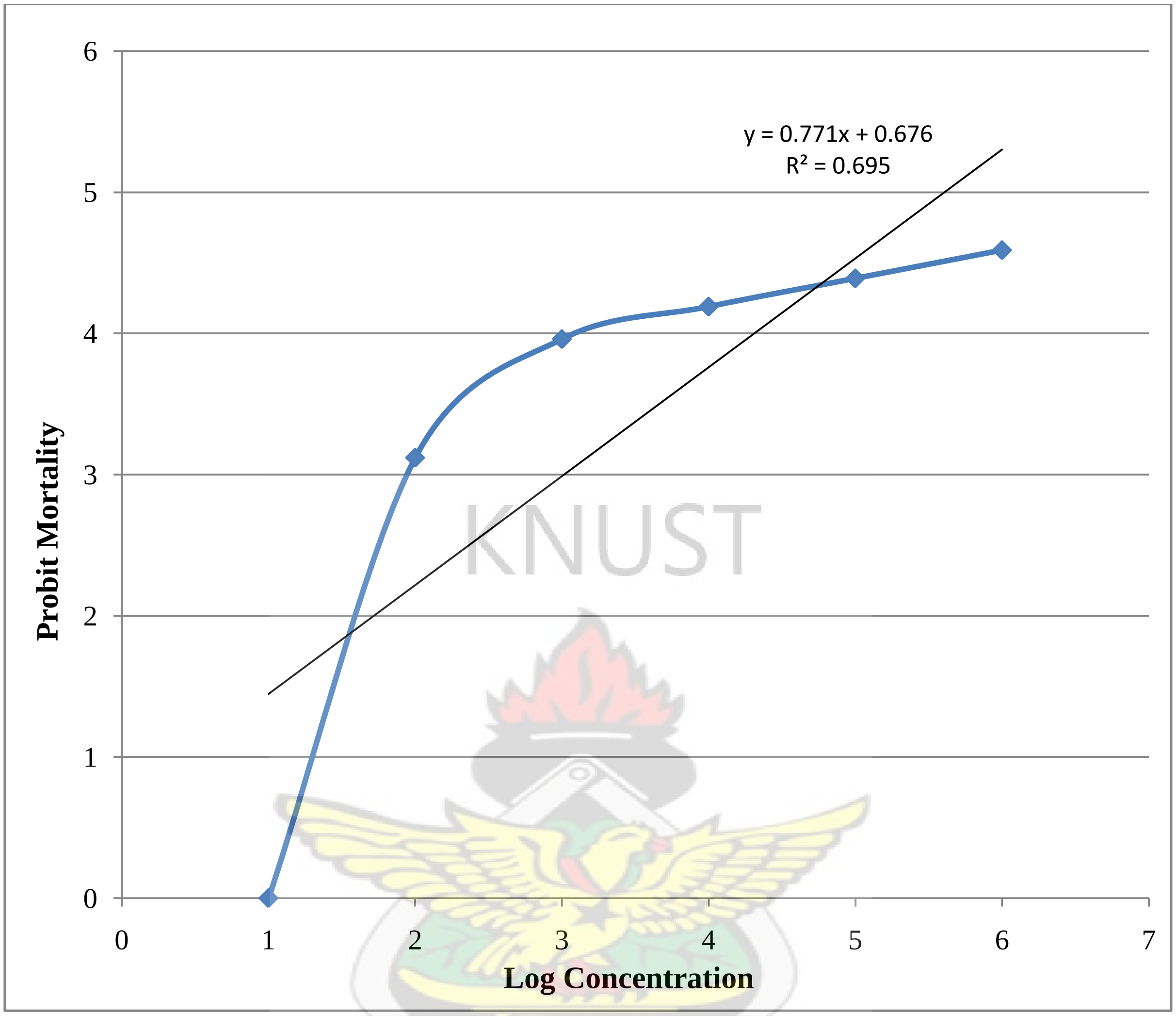


Figure 4.18: Relationship between Log Concentration and Probit of Response

CHAPTER FIVE

5.0 DISCUSSION

5.1 QUESTIONNAIRE SURVEY

Pests and diseases pose greater challenges in agro ecosystem. The damage caused by pest has led to farmers using pesticides. It was estimated by Dinham (2003) that 87% of farmers use chemical pesticides to control pests and diseases on vegetable crops in Ghana which is in agreement with findings in this survey. A vast majority of farmers (78%) had either basic education or no education hence could not read labels on the chemical containers. Due to the high level of illiteracy among farmers in the study area, they generally classified any chemical which kills insects as “poison”. Interviewers asked farmers to bring out the kind of poison they commonly used. The common insecticides mentioned by the farmers were lambda (24.2%), controller (22%), karat (19%), kombat (24%) and rocky (10%). The survey also revealed that sunphosate, condemn, asaasewura and power were the prominent herbicide constituting about 98% of all herbicides in use. The questionnaire survey also revealed that majority of the respondents (74%) were male adults which is indicative of male dominated profession between the ages of 18 and 60 years.

According to the survey and during the interview, it was observed that most farmers did not normally follow the manufacturer’s formula for mixing pesticide for spray. Some farmers used measuring cans and bottles to measure pesticide using different ratios based on perception. Others used the dose or rate contained in the pack, can or bottle for a given volume of water or for a given acreage respectively. Observations made at most of the farms revealed that most farmers increased the concentration by adding more of the chemical substance or added other chemicals to boost potency. It was evident in the survey that some farmers added ammonia (fertilizer) to herbicides solution to increase potency and to achieve quicker results.

It was also revealed that chemical pesticides were usually sprayed in combination with other pesticides and the efficacy of one might have catalyzed or masked the efficacy of others in the mixture. This procedure was according to the farmers adopted to achieve rapid knockdown of pests. This idea is questionable (Medina, 1987) at least as practiced, because the combinations used were indiscriminate. The practice of using indiscriminate combinations of pesticides, particularly of insecticides, may have contributed to the increase rate of insect pest infestation resulting from their resistance (Biney, 2001). This practice defies some of the basic principles of insecticide management. Metcalf (1980), in his recommendation of strategies for pesticide management, indicates that the use of mixtures of insecticides must be avoided, since this practice generally results in the simultaneous development of pest resistance to pesticides.

Determination of the right concentration of the pesticide solution before application on the farms was sometimes done by using the tongue to taste the concentration. (The sprayers would dip a finger into the solution and the sample picked is dropped unto the tongue). The level of concentration is normally determined by the sharpness of sensation felt on the tongue. By this, a very sharp sensation means normal concentration, while blunt sensation means low concentration. This method of testing concentration is very hazardous to human health due to non-compliance of safety regulations. Non-use of personal protective equipment such as respirators, rubber gloves and water resistant clothes was not a bother to many of the farmers.
This study further revealed that most farmers/applicators seldom wear inappropriate clothes during pesticide spraying exposing the body to pesticide. The vast majority of the farmers

(58%) did not use PPE at all while 29% used some form of PPE exposing them to pesticide hazards.

According to the survey the main route of exposure to pesticide was through the skin (48%), nostrils (33%), the eyes (13%) while through the mouth accounted for 7% causing unfavorable reactions to farmers such as itching, burning skin, blurred vision and respiratory problems. Farmers were exposed to the chemicals because of non-use of personal protective equipment (PPE) especially cover cloths during spray and sometimes leakages from knapsack spray cans. Spraying during windy conditions also cause drifting of the chemicals to unapproved areas.

The data collected also revealed that 60% of the farmers had fallen sick ranging from general body weakness, dizziness, headache, skin diseases immediately after pesticide application.

The survey also revealed that farmers sprayed the same very wide range of pesticides (broad spectrum) on all their crops killing non-target organism such as pollinators and soil organisms. Some farmers sprayed the recommended and approved insecticides such as lambda super on their crops while others used the banned insecticides such as DDT. This practice has implications for public health. For instance, endosulfan has a restricted use in Ghana that does not include vegetables (it has only been registered by EPA Ghana for use on cotton, yet it is used on vegetables. DDT on the other hand has been banned from being used in Ghana nevertheless, some farmers secretly use on their vegetable farms. There are no obvious indications of public health problem as a result of the misuse of these products, the risk is clearly evident. The use of endosulfan and DDT on vegetables in Ghana is worrying given the toxicity and persistence in the environment.

The survey ascertained that pesticides are readily available in agricultural retail stores in Akumadan, and those responsible for retailing them (pesticide dealers) have adopted the extensive practice of hiding the banned products and intentionally selling to ignorant users.

The survey also revealed that 82% of the respondents dispose pesticide containers on the farms after applications which sometimes spill the residue in the container onto the land and these may leach to contaminate streams and waters near by. It was also revealed that most of the farmers reused the pesticide empty containers as drinking cups after slashing into two exposing some farmers to health hazards.

5.2 THE FIELD WORK

When pesticides are used on bare soil or with very little plant cover, as was the case after one and a half months of planting the seedlings, the estimation is that nearly the total amount of pesticides applied will enter the soil (Kokta and Rothert, 1992). The field work was undertaking during the lean farming season characterized by high temperatures and low rainfall along the Akumadan River bank where soils were constantly damp.

Earthworms were affected at very low dose exposures to pesticides when applied at 2-week intervals in a laboratory study (Springett and Gray, 1992). It therefore seems that the earthworms, although not exposed to the pesticide continuously for extended periods, could be affected by the exposure. Handy (1994) stated that chronic and repeated sublethal episodes of contamination may have equally important detrimental effects on organisms. The establishment of causal relationships between population density of earthworms and degree of pesticide exposure in field is difficult due to the fact that a variety of environmental influences could be responsible for the observed condition (Reinecke and Reinecke, 2004).

During the field work, higher relative abundance (45%) of earthworms were found in the control farm (pristine area) distanced from the test area, where the vegetation and soil types were the same but drier and less disturbed with lower concentrations of pesticides drift. The low earthworm relative abundance found consistently in the test field during the surveys could be the result of historical farming practices, prevailing climatic conditions and soil properties (Curry, 2004).

Earthworms are very sensitive to moisture conditions in their substrates (Reinecke and Venter, 1987; Viljoen and Reinecke, 1998). If this was a more important limiting factor, a higher density of worms would have been expected in the test field than in the control field as the crops were under regular irrigation and the soil had higher moisture content than in the control area. This is a further indication of the possible damaging role that pesticides had played to affect earthworm relative abundance. Similar findings were observed by other Authors in course of their studies (Clements et al; (1991), Chan; (2001) and Curry; (2004).

It is also a known fact that earthworm populations are usually smaller in arable land than in physically undisturbed habitats (Lee, 1985). As the control area was well established and the soil has not been ploughed or disturbed recently in any way, physical disturbance could not have been an important limiting factor at present, although the soil has a long previous history of disturbance due to cultivation, the possibility therefore exists that spraying of pesticides could have been an important factor causing lower abundance of earthworms in the test than in control areas. According to Stenersen (1979) long-lasting exposure under field conditions could have lethal effects on earthworms. During the study the pesticides were not distributed homogeneously in the soil. Avoidance of exposure could therefore occur if the pesticide could be sensed by the earthworms (O'Halloran *et al*., 1999).

5.3 LABORATORY ANALYSIS

The results of the study reported no mortality on the control worms, while varying degrees of mortality were reported in the test concentrations. This is a clear indication that the pesticide could be regarded as possible cause of death of the earthworms.

The highest mortality of 34% and 27% were found at the highest concentrations of 0.64 and 0.32ppm respectively suggesting dose-dependent mortality and concentration graded lethality while the lowest mortality of 3% was found at the lowest concentration of 0.04ppm.

It was observed also that the earthworms exposed to the highest concentration of 0.64 remained at the bottom of the test containers whereas the lowest concentration and control earthworms exhibited excellent burrowing movements in the earth pot and exhibited no other extraordinary behavior.

Morphological changes such as constriction and swelling started appearing in the anterior regions of exposed worms within one week of exposure and degenerative changes appeared at the posterior end of the exposed earthworms after the two weeks period of exposure.

From the graph (Fig. 4.16), the coefficient of regression (R^2) shows that 90% mortality of earthworms could be due to the concentrations of the chemicals they were exposed to. From this, an increased concentration of 1ppm is associated with an average corrected mortality of 9 as shown in Fig. 4.16. From Figures 4.16 and 4.17, R^2 of 0.98 and 0.90 respectively show a very strong correlation between concentration and mortality as about 90% of variations in mortality is explained by the regression of mortality on concentration.

In a similar manner, the probit-log concentration graph which is a direct transformation of log-percentage mortality graph (Fig. 4.17) shows a similar R^2 of about 0.70.

Probit Analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response (Finney and Stevens, 1948).

The log-probit scale therefore yielded the LC_{50}. The LC_{50} or statistically-derived dose that is lethal to 50% of the test population was helpful to compare the toxicity between the various concentrations.

5.4 IMPORTANCE OF FINDINGS

Findings of this study show apparent negative effects of the application of pesticides on non targeted organisms including human beings. Therefore, in-depth follow-up studies to establish the actual effect of chemical on non targeted organisms is recommended in order to guide farmers on the use of environmentally friendly pesticides in order to safeguard the non targeted organisms in the environment.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY AND CONCLUSION

The questionnaire data generated was helpful to assess the impact of pesticide on the population and toxicity to the nontarget organisms. It was evidenced that the use of tongue to test concentrations and non compliance of safety regulations and use of personal protective equipment was harmful to health. Most farmers did not also follow the harvest time interval by the manufacturer and harvest the vegetables as soon as sprayed causing health problems to consumers.

The spraying of broad spectrum pesticides on crops killing non-target organism such as pollinators and soil organisms is a threat to the agro ecosystem. The use of banned pesticides such as DDT on vegetables in Ghana is worrying given the toxicity to nontarget organisms and persistence in the environment. The reuse of the pesticide empty containers as drinking cups exposes the farmers to health hazards.

This study has also demonstrated that mortality increased with concentration and the length of exposure to pesticides in the soil. This suggests that mortality is associated with concentration and exposure duration. The study found low earthworm densities with lower numbers in the test farm than in the control area. Factors such as soil disturbance and moisture did not seem to be limiting, it can therefore be concluded that the presence of the pesticides in the soil could be an important factor causing low earthworm numbers.

The two weeks field simulated laboratory test was more suitable for observing the actual pathological changes that were apparent and close to reality on the test farm. The results of the laboratory study reported no mortality in the control test, while varying degrees of mortalities were reported in the test concentrations. This is a clear indication that pesticide could be regarded as possible cause of death of the earthworms on the farms.

Comparing the data obtained on the field to that in the laboratory, this study established a direct relationship between concentration and mortality. These results lend support to the results of several studies that there is a direct relationship between concentration of the chemical and mortality it causes to the worms.

6.2 RECOMMENDATIONS

1. The use of broad spectrum pesticides should be discouraged as such non selective insecticide application is highly deleterious to the environment. Pesticides should therefore be manufactured to target specific pest to minimize the devastating effect of non target organisms
2. Farmers should be educated periodically by the stakeholders such as the Environmental Protection Agency (EPA), Ministry of Agric (Extension Officers), the Chemical Sellers Associations and other NGO_s on the hazards associated with handling, mixing and use of pesticides so as to minimize the hazardous effects of pesticides application.
3. Further studies should be carried out to determine the reaction of the active ingredients to weather and the effect of climate on pesticides imported to the country since environmental conditions contribute to the risks and hazards associated with pesticides.
4. Efficient laws should be enforced by the stakeholders (EPA and Agric Ministry) to ensure that banned and unregistered pesticides are not imported and used. The Environmental Protection Agency of Ghana should also enforce the Biopesticide Registration and Risk

Assessment Guidelines to facilitate greater use of biologically based safe pesticides with the aim of contributing to a reduction in the use of toxic synthetic chemical pesticides

5. Awareness should also be created regarding the ill-effects of pesticides use and use of personal protective equipment by farmers should be ensured by the Government Agencies such as EPA, MOH/MOA as well as chemical manufacturers.

6. Integrated Pest Management (IPM) which manages pest populations by harmonizing control methods such as natural enemies, pesticides and cultural practices should be encouraged. The purpose of IPM is not to eradicate or remove the pest population completely, but to manage pest populations so that economic damage and harmful environmental side effects are minimized.

7. Study on the harmful effects of chemical pesticides on farmers should be undertaken by conducting regular medical tests such as blood and urine screening to determine levels of pesticide in the blood/urine as well as field measurements of chemical concentrations at the working environments.

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APPENDICES

APPENDIX A

QUESTIONNAIRE TO ASSESS THE ECOLOGICAL IMPLICATIONS OF PEST MANAGEMENT IN A FOREST AGROECOSYSTEM. A CASE STUDY OF THE RELATIVE TOXICITY OF SOME PESTICIDES USED FOR THE CONTROL OF PEST IN AKUMADAN, GHANA.

This questionnaire should be completed for each farmer exposed to pesticides in agro ecosystem

1.0 Person exposed (identity should be checked)

Sex: □ male □ female □ age ……………………….

If age unknown: □ child(<14yrs) □ adolescent (14-19 yrs) □ adult (>19 yrs)

2.0 Educational background □ Basic □ Secondary □ Tertiary □ None

3.0 (a) Which pesticides do you use the most?

Please specify…..……………………………………….

3.0 (b) Which weedicide do you use the most?

Please specify ……………………………………………………………………….

4.0 Type of formulation being used (check one of the following on the container label)

□ Emulsifiable Conc. (EC) □ Wettable Powder (WP) □ Dustable Powder (DP)

□ Water Soluble Powder (SP) □ Ultra Low Volume (ULV) □ Tablet (TB)

□ Granular (GR) □ other, please specify …………………………………………..

5.0 Under which of the following weather conditions is it not advisable for you to apply pesticides?

□windy □rainy □hot

Any other condition, please specify…………………………………………………

Explain why? ………………………………………………………………………..

6.0 (a) Have you ever had any unfavorable reaction after pesticide application?

□Yes □No

If yes, what was your most unfavorable reaction? (check one or more of the following)

□dizziness □headache □blurred vision □ excessive sweating

□ hand tremor □ convulsion □ staggering □ narrow pupils/miosis

□ excessive salivation □ nausea/vomiting □ others, specify…………….……………

6.0 (b) Which type(s) of pesticide(s) gives you such unfavorable reaction?

………………..

7.0 If you have ever had any unfavorable reaction after pesticide use, which part of your body do you think was the route of exposure? (more than one if applicable)

□ mouth □ skin □ eyes □ nostrils

Others, please specify………………………………………………………………..

8.0 How often do you use protective clothing during pesticide application?

□ always □not always □not at all

Please explain why ……………………………………..……………………………

9.0 If you always use protective clothing, which of the following is/are often used. (check one or more of the following):

□gloves □ overalls □ eye glasses □ respirator □face mask □boots

□ shoes □long-sleeves shirt □trousers

10.0 Have you ever heard of any human sickness resulting from pesticide use in this area?

□Yes □No

If yes, please specify the type of sickness …………………………………………….

....

11.0 Have you ever heard of any human death resulting from pesticide use in this area?

□Yes □No

If yes, when and where did it take place……………………………………………….

12.0 Have you ever heard of any animal death resulting from pesticide use in this area?

□Yes □No

If yes, please indicate the type of animal …………………………………….…….

13.0 How do you dispose off used pesticide containers?

□ bury □ burn □ throw on the ground □ throw in water bodies

□ use as drinking cups □ use as food containers

Others, please specify …………..…………………………………………………….

Why? ………………………………………………………………………………….

…………………………………………………………………………………………..

14.0 How often do you wash your hands after pesticide application?

□often □not often □not at all

Other, please specify …………………………………………………………………

15.0 Does pesticide affect non target organisms such as honey bees and earthworms during application?

□ yes □ no

If yes, in what way, please specify…………………………………………………….

APPENDIX B

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) SUBSTANCES CONTROL ACT AND THE FEDERAL INSECTICIDE, FUNGICIDE AND RODENTICIDE ACT. ECOLOGICAL EFFECTS TEST GUIDELINES (EARTHWORM SUBCHRONIC TOXICITY TEST)

(A) SCOPE

This guideline is one of series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act and the Federal Insecticide, Fungicide and Rodenticide Act.

(B) PURPOSE.

This guideline is intended for use in developing data on the toxicity of chemical substances and mixtures (‘‘chemicals’’) subject to environmental effects test regulations under Toxic Substances Control Act (TSCA). The guideline sets forth the procedures and conditions for conducting toxicity tests. The EPA use data from these tests in assessing hazard of chemicals to earthworms in the soil environment.

(C) DEFINITIONS.

The following definitions apply:

Artificial soil means a defined dry weight mixture of 68 percent of No. 70 mesh silica sand, 20 percent kaolin clay, 10 percent sphagnum peat moss, and 2 percent calcium carbonate. These ingredients are weighed and mixed in the above proportions and moistened to 35 percent (by weight) with deionized/distilled water.

Behavioral symptoms are indicators of toxicity to earthworms such that a distinct difference in position in the test container can be identified, e.g. below surface or on the surface, writhing on the surface, stiffened and shortened on the surface or elongated and pulsing or inactive below surface in a ball.

Clitellum means a glandular portion of the anterior epidermis, appearing as saddle-shaped or annular, usually differentiated externally by color.

Culture means the animals which are raised on-site or maintained under controlled conditions to produce test organisms through reproduction.

EC50 means that test substance concentration calculated from experimentally-derived growth or sublethal effects data that has affected 50 percent of a test population during continuous exposure over a specified period of time. LC50 also means experimentally derived concentration of test substance that is estimated to kill 50 percent of a test population during continuous exposure over a specified period of time.

Lowest observed effect concentration (LOEC) means the lowest treatment (i.e., test concentration) of a test substance that is statistically different in adverse effect on a specific population of test organisms from that observed in controls.

Mature or adult worms mean a condition of the worm exhibiting a clitellum in the anterior of the body.

Mortality means the lack of movement by the test organism in response to a definite tactile stimulus to the anterior end. Also, because earthworms tend to disintegrate rapidly after death, the absence of organisms in the enclosed soil test container is considered to mean death has occurred.

No observed effect concentration (NOEC) means the highest treatment (i.e. test concentration) of a test substance that shows no statistical difference in adverse effect on a specific population of test organisms from that observed in controls.

Pathological symptoms mean toxic effects, such as surface lesions and midsegmental swellings or general ulcerated areas on the surface of the earthworm.

Test mixture means the test substance/artificial soil mixtures which the earthworms are exposed to during the test.

Test substance means any compound used in artificial soils spiked for laboratory testing of toxicity.

(D) TEST PROCEDURES

(1) Test species Selection.

The test species for this test is the earthworm *Eisenia fetida* andrei (Bouche). The species identity of the test organism should be verified using appropriate method.

(i). Age and condition of earthworms.

Adult earthworms should be used. Earthworms used in toxicity tests should come from a verifiable source. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test should have originated from the same population (culture). All newly acquired earthworms should be quarantined and observed for at least 14 days prior to use in a test. Earthworms should not be used if they have been under stress from too much or lack of moisture, excessive or inadequate food, temperature, pH variation, or crowding. Any of these conditions will produce earthworms that may not be healthy.

(ii). Preparation.

Sufficient numbers of earthworms should be harvested and sorted to insure that healthy individuals are used for the test. Any animals that appear to be injured should not be used in the test and must be discarded.

(iii). Acclimation of test earthworms.

Adult earthworms should be handled with care. Earthworms should be held for a minimum of 7 days in uncontaminated soil at the test temperature prior to testing.

(iv). Feeding.

The earthworms are not fed during the test period.

(2) Number of test animals

A minimum of 30 earthworms exposed to each test concentrations and control should be tested. Ten earthworms per container of artificial soil should be placed in replicates for each concentration and control. The distribution of individual earthworms among the test chambers should be randomized.

(3) Medium preparation.

(i). For each concentration tested and controls, enough artificial soil must be prepared by recipe to yield 270 g of artificial soil (wet weight) per replicate.

(iii) An appropriate amount of high purity water should be added to the artificial soil and mixed to raise the artificial soil moisture level to 35 percent by weight.

(iv) Appropriate portions of the artificial soil should be mixed thoroughly with appropriate amounts of test substance to yield replicates for each test concentration. Each test mixture is divided into equal quantities as determined by weight. Each portion is placed into a separate container and represents one replicate for exposing 10 earthworms at the same concentration.

(vi) Prior to the addition of earthworms, a 10–g sample should be removed from each replicate to measure pH and test concentrations.

(4) Definitive test.

(i) The test should be designed to determine a concentration- mortality curve and estimate the respective LC50 values and 95 percent confidence intervals.

(ii) The toxicity test should use earthworms which are maintained in direct contact with artificial soil allowing earthworms to ingest contaminated soil *ad libitum*.

(iii) Test concentrations should be chosen in a geometric series in which the ratio should be between 1.5 and 2.0 mg/kg (e.g., 2, 4, 8, 16, 32, and 64 mg/kg). All test concentrations should be based on milligram of test chemical (100 percent active ingredient) per kilogram of artificial soil (airdry weight).

(v) The living earthworms should be placed on the surface of the medium and the container capped and secured without making an airtight seal.

(vi) Any changes in soil temperature should not exceed 3°C per day or 1°C per hour. Earthworms should be held for a minimum of 7 days at the test temperature prior to testing.

(vii) Every test should include a control consisting of uncontaminated artificial soil.

(E) DATA REPORTING AND EVALUATION

The reporting of test data should include the following information:

(1). Description of equipment and test method

(i) Test Background including the name of the sponsor, testing laboratory, principal investigator, and dates of testing.

(ii) A detailed description of the test chemical including its chemical identification (trade name, common name), source, composition (identity and concentration or major ingredients and major impurities), known physical and chemical properties, empirical formula, water solubility, vapor pressure, manufacturer, method of application, and any carriers or other additives used and their concentrations. The volume or mass of any carriers should be reported. An exact description of how the test substance has been mixed into the artificial soil.

(iii) Detailed information about the earthworms used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, and culture method.

(iv) A description of the test situation, especially if there was a deviation from this test guideline as described above in soil preparation, addition of the chemical, culturing of the test species, lighting, pH, temperature, replicates, or the number of organisms per container.

(v) A description of the test container used, its size, volume and weight of soil used in each container, number of test organisms per container, number of test containers per concentration, conditioning of the test container, description of the method of test chemical introduction into the test medium (e.g., as a powder), stock solution used or not, and time between mixing of the stock solution and introduction of the earthworms.

(vi) The concentration in artificial soil at the beginning of the test and the actual concentrations of the test chemical (if measured) in the soil before (day 0), during (day 7, 14, 21) and upon the conclusion of the test (day 28) and the dates the analyses were performed.

(vii) The number and percentage of organisms that were killed or showed any adverse effects at each test concentration, including controls, in each test container at each observation period.

(viii) Concentration response curves fitted to mortality data at 7, 14, 21, and 28–day periods.

(x) The LC50 values and the 95 percent confidence limits using the mean measured test concentration and the methods used to calculate the LC50. The probit technique should follow the methods described by Finney. Appropriate statistical methods (e.g. one-way analysis of variance and multiple comparison tests) should be used to test for significant differences between treatments

(2). Test results.

(i) Death is the primary criterion used in this test guideline to evaluate the toxicity of the test substance.

(ii) In addition to death, weight loss, behavioral symptoms and pathological symptoms should be recorded.

(iii) Each test and control chamber should be checked for dead or affected earthworms and observations recorded 7, 14, 21, and 28 days after the beginning of the test or within 1 hour of the designated times. Missing earthworms should be considered to have died.

(iv) Mortality is assessed by emptying the test medium on a glass or other inert surface, sorting earthworms from the test mixture and testing their reaction to a gentle mechanical stimulus. Any adverse effects (e.g. weight loss, behavioral or pathological symptoms) are noted and should be reported. The medium is returned to each container.

(v) The test result is to be unacceptable if:

- More than 20 percent of control organisms die

- The total mean weight of the earthworms in the control containers declines significantly during the test (i.e., by 30 percent).

(vi) Mortality is checked and recorded at days 7, 14, 21, and 28.

(vii) The mortality data should be used to calculate LC50 values and their 95 percent confidence limits, and plot concentration-response curves

APPENDIX C

NOTES ON STATISTICAL METHODS

Statistical evaluation of the difference between pairs of means was generally based on analysis of variance. The Bartlett's equal variance and *F*-test was used on some data.

Analysis of Variance (ANOVA)

The complete analysis of variance table is cumbersome and can be substantially reduced without loosing any of the information embodied in it.

If values for variance – ratio (V-R) and residual mean square (RMS) are known the complete table may be reproduced as follows:

MS (mean squares) = V-R × RMS

SS (sum of squares) = MS × df (degree of freedom)

The total sum of squares (SS) is found by summation of the above values.

APPENDIX D

FINNEY'S TABLE (1952)

Transformation of Percentages To Probit

%	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	4.29	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	5.25	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	0.07	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

APPENDIX E

CALCULATION OF LC50 RELATING TO PROBITS AND LOG-DOSE USING BEHRINES AND KARBER FORMULA

The procedure for computing the LC50 using probit analysis is as follows:

1. Calculate mean dead earthworms between two successive doses (A)
2. Calculate dose difference between two successive doses (B)
3. Multiply A and B
4. Divide the result of AB by the total number of earthworms (N)
5. The LC_{50} value is determined by subtracting the value of AB/N from the largest dose (0.64ppm)
6. The LC_{50} is calculated using Behrens and Karber (1953) equation:

$$\text{LC50} = \text{Largest Dose} - \sum \frac{\text{A} * \text{B}}{N}$$

Where:

A = mean dead earthworms between two successive doses.

B = dose differences between two successive doses.

N = the total number of earthworms.