INSECTICIDE USE PATTERN, RESIDUES IN SOIL AND WATER AND *KJDR* RESISTANCE IN *ANOPHELES GAMBIAE* S.L. ON RICE FARMS IN OKYEREKO, CENTRAL REGION.

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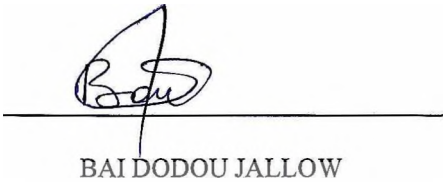
DECLARATION

This is to certify that this thesis is the result of research undertaken by Bai Dodou Jallow towards the award of Master of Philosophy (MPhil) in Entomology in the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Legon.



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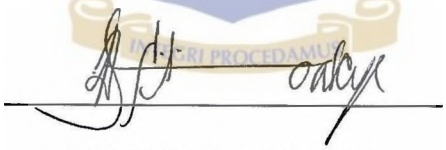

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DEDICATION

To my dad, Alh Cherno Jallow (late) and mum, Aja Haddy Khan, my lovely wife Mrs Kadijatou Jallow, daughter Fatoumatta Jallow and to the entire Jallow family in Kerr Mama.

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ABSTRACT

Indiscriminate use of insecticides in controlling insect pests of agricultural crops has been implicated in the development of resistance in disease vectors such as the *Anopheles gambiae* s.l Giles (Diptera: Culicidae) populations that breed within farming areas. Since vector control strategies are insecticide based, it is important to study insecticide resistance in farming areas such as irrigation sites where vectors are breeding. The aim of the study was to investigate insecticide use pattern among rice farmers in Okyereko, Central Region of Ghana, determine residues level in soil and water samples from mosquito breeding grounds and to study *kdr* resistance in *An. gambiae* populations on rice farms in the area. A questionnaire based survey was used to investigate the pattern followed in the use of insecticides. The survey results showed that farmers were using only pyrethroids (permethrin and lamdacyhalothrin) against stemborers and caseworms which are the key rice pests in the area. The general pattern of using insecticide is plagued with many problems due to lack of adequate knowledge and inappropriate equipments for proper insecticide use. Insecticide residues level in soil and water samples were determined using gas chromatograpy-mass spectrometry. Varying levels of lamdacyhalothrin, permethrin and cypermethrin were detected in both soil and water samples suggesting that these environments were contaminated with these insecticides. Susceptibility status of *An. gambiae* s.l to malathion propoxur, DDT, permethrin and deltamethrin was determined using WHO bioassay test kit. The population was found to be susceptible to malathion and propoxur but resistant to DDT, permethrin and deltamethrin. High knockdown times were recorded for the wild population relative to the susceptible strain. Molecular studies revealed that *An. gambiae* s.s was the dominant

species in the area and its M form was found to be more prevalent as all tested individuals were discovered to be *An. gambiae* s.s M form. *Kdr* mutation gene was detected in all tested *An. gambiae* s.s individuals suggesting that *kdr* mutation is one of the main resistance mechanisms employed by *Anopheles* mosquitoes in the area. The indiscriminate use of insecticides in the area, the residue levels in soil and water samples from breeding grounds, the resistance ratios and the high presence of *kdr* gene is suggestive that, the probable misuse of insecticides in agriculture is strongly contributing to the development of resistance in *Anopheles* mosquitoes breeding in the area.

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LIST OF ABBREVIATION

Bp	Base pairs
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide phosphate
EDTA	Di sodium ethylene diamine tetracetate. $2H_20$
EtBr	Ethidium Bromide
ETOH	Ethanol
H_20	Water
M	Molar (moles per litre)
mM	Millimolar
jil	Microliter
KAc	Potassium acetate
pH	LoglO[H+]
rpm	Revolution per minute
RNase	Ribonuclease
s.I	*sensu lato*
s.s	*senso stricto*
Tm	Melting temperature
g	Gram
mg	milligram
Hg	Microgram
An	*Anopheles*
SPE	Solid Phase Extraction
Kdr	knockdown resistance
KDT	knockdown time

CHAPTER ONE

GENERAL INTRODUCTION

1.0 Introduction

Over the last 60 years there has been an exponential increase in human population in most parts of the world. Due to this population increase the demand for food has risen, forcing farmers to engage in intensive agriculture in order to meet the needs of the ever increasing population. Farming areas have increased in size and farmers have tended to specialize in the cultivation of one or a few crops. This system of farming (monoculture) has created a suitable environment for the development of many pest species and destroyed the natural balance of ecosystems. Natural enemies such as parasitoids and predators have been affected and insect species which were under natural control have now become key pests.

In Ghana, increase in urbanization and the growth of the middle class have led to increase in the consumption of rice (JICA, 2006). Rice farmers such as those in Okyereko (a rice irrigation area), are currently challenged by the ministry of agriculture to increase domestic rice production in order to reduce the amount of imported rice (JICA, 2006). They have therefore resorted to intensive production and the use of new high yielding varieties. These new varieties are more susceptible to damage by insect pests such as *Nymphula depunctalis* Guenee (Lepidoptera: Pyralidae) that attack irrigated rice. In a bid to maintain productivity, the farmers have resorted to the use of chemical control to control these insect pests.

The success of chemical plant protection is convincing, optimum yield is obtained and the quality of farm produce is improved (GTZ, 1979). Farmers thus apply insecticides in order to boost production. However, due to the lack of adequate knowledge and resources, indiscriminate application and misuse of insecticide is likely to occur. Developing countries use only 20% of pesticides in the world (Pimental, 1996). The high proportion of human poisonings and deaths occurring in these countries may reflect the existing conditions of inadequate enforcement of standards, poor labelling of pesticides, lack of safe handling and application, poorly implemented controls, illiteracy and insufficient knowledge of pesticide hazards by farmers (Pimental 1996). Aside from the effects that chemical insecticides have on target organisms, they also have non-target effects that include resistance development in vectors of diseases such as mosquitoes which breed within and around the farming area. Additionally, they cause insecticide residue accumulation in the environment (soil, water and air).

Irrigated rice fields are mostly located on lowland areas close to water bodies. The pools of water found in furrows, potholes within the fields and water bodies located around the farms are excellent breeding sites for mosquitoes. Some of these sites are sprayed directly whenever crops are treated with herbicides and insecticides. Other sites are reached by these pesticides via various means through run-off, wind drift and leaching. When pesticides are applied to protect crops from pests and diseases, only around 15% of the preparation hits the target. The rest is distributed in the soil, air and water (Varca, 2002).

Insect vectors of diseases such as the *Anopheles gambiae* s.l that breed within and around insecticide polluted areas are exposed to these chemicals during early stages of their life cycle and those that survive are selected for resistance and they pass this characteristic on to subsequent generations.

Strong evidence implicating agricultural insecticides in the selection of resistance in insect vectors is shown in the resistance to compounds which had never been used for public health, but were employed in agriculture (Mouchet, 1988). Secondly, the level of resistance in some insect vectors has been linked to the quantity of the compound used in the same area against crops pests (Mouchet, 1988). Larval exposure to insecticide contaminated agricultural run-off from cotton and rice fields near mosquito breeding sites has led to the development of resistance in mosquitoes (Akogbeto *et al.,* 2006).

Insecticide resistance is an inherited characteristic. In a population of resistant insects the majority of individuals are able to survive doses of insecticide that would kill the majority in a susceptible population of the same species. In insects such as the *Anopheles* mosquitoes, resistance can occur relatively faster due to their short life cycles (Brogdon and McAllister, 1998). Insecticide resistance in *Anopheles* has largely been characterized as being biochemically based; and two major forms are recognized: target site insensitivity and detoxification enzyme-based resistance (Brogdon and McAllister, 1998).

Malaria is transmitted through the bite of a female *Anopheles* mosquito which is infested with the *Plasmodium* parasite. It is estimated that over 41% of the world population live in areas with malaria risk, with the greater majority living in sub-Saharan Africa (WHO, 2006b). There are 300-500 million cases of malaria a year worldwide leading to 1.2 to 1.6 million deaths, 80-90% of which occur in Africa (WHO, 2008). In Ghana, malaria is one of the major health problems. The disease is hyper-endemic and accounts for over 40% of all outpatient cases seen in health institutions and 44.1% of all clinical attendance. It is responsible for 7-8% of all certified deaths and ranked as the fifth most common cause of death in the 0 to 4 years age group (Ghana Demographic and Health Survey, 2003).

An important aspect of reducing the malaria burden lies in vector control which targets various stages of the life cycle and also the ecology and behaviour of the vector. Many vector control strategies are chemical based. Insecticide treated bed nets and indoor residual spraying with chemical insecticides are used as the principal vector control options (WHO, 2008). At present, pyrethroid insecticides are the only option for impregnating bed nets for malaria control (WHO, 2006a). Pyrethroid-impregnated bed nets have been shown to be an effective means of control with protection lasting 6-8 months. They have been shown to provide a remarkable degree of protection against malaria in Africa (Curtis *et al.,* 2003). Randomized control trials carried out in Ifakara, Tanzania revealed that the use of insecticide treated bed nets had a protective efficacy of over 62% on the prevalence of parasitaemia (Abdulla *et al.,* 2001). In Kassena Nankana district of Ghana, the use of permethrin impregnated bed nets was associated with 17% reduction in mortality in children aged 6 months to 4 years (Binka *et al.,* 1996).

However, resistance to this group of insecticides has been reported in Burkina Faso, Cote d'Ivore, (Chandre *et al.,* 1999a, b), Ghana (Adasi *et al.,* 2000) and Kenya (Vulule *et al.,* 1999; Ranson *et al.,* 2000). The development of resistance by *An, gambiae* (the principal malaria vector in sub-Saharan Africa) to insecticides used in vector control programs is a cause for alarm. It has the risk of rendering complete vector control programs meaningless thereby increasing the burden already created by the disease. Insecticide resistance is assumed to increase the likelihood of mosquito-borne disease transmission by increasing the vector population size and allowing mosquitoes to live longer in the presence of insecticides (McCarrol and Hemingway, 2002).

1.1 Rationale

Increase rice production in irrigated areas is challenged by the presence of a variety of insect pests which have the capacity to markedly reduce the quantity and/or quality of production. Farmers, therefore, are compelled to control these pests and they normally resort to the use of chemical insecticides. These insecticides in addition to the effects they have on target pests also exert a selection pressure on vectors of diseases such as mosquitoes that breed within the farming area. This consequently leads to the development of resistance to the insecticides used and probably other insecticides having a similar mode of action. Work conducted in Burkina Faso by Diabate *et al.* (2002a) shows that agricultural use of insecticides is involved in the selection for resistance to permethrin and DDT in field populations of mosquitoes.

Resistance development by *Anopheles* vectors to insecticides,the main weapon recommended by WHO and employed by national institutions in the fight against malaria, has exacerbated the fight against the disease. Therefore, understanding the nature and causes of vector resistance to insecticide currently in use cannot be overemphasized. Studies conducted by Achonduh (2005) and Ben-Mahmoud (2008) revealed the presence of elevated acetylcholinesterase and oxidase activities and the presence of *kdr* resistance gene in *Anopheles gambiae* s.l populations in cabbage growing areas in the Accra metropolis. Residual bioactivity was also detected in soils from the fields and in run-off water. In Anloga, Volta Region a positive correlation was observed between residue levels, residual bioactivity in the soils and water samples and resistance levels in *An. gambiae* s.l. populations breeding within shallot farms (Obeng, 2007).

It is therefore important to extend this study to other regions of the country especially in irrigation schemes and in areas of high insecticide use on crops such as rice. This should be done in order to obtain more information that can be used as support base for planning a national malaria vector control strategy.

1.2 General Objective

This study was carried out to determine the pattern of insecticide use among rice farmers in Okyereko, analyze residues in soil and water on farms and determine the level of *kdr* resistance of *Anopheles gambiae* s.l populations in the area to insecticides.

1.2.1 Specific Objectives

The specific objectives were to:

1. Conduct a survey on insecticide use pattern among rice farmers in Okyereko.

2. Morphologically identify *An. gambiae* s.l species

3. Determine susceptibility status of *An. gambiae* s.l to the following synthetic insecticides: DDT, permethrin, deltamethrin, propoxur and malathion using WHO test kits.

4. Identify *An. gambiae* s.s into M and **S** forms and determine the prevalence of *kdr* mutation gene within the tested *An. gambiae* s.s population using PCR based methods

5. Determine the presence of insecticide residues and levels in mosquito breeding waters and in soils from breeding sites.

6. Establish a relationship between insecticide residual levels and resistance development in *An. gambiae* s.l.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rice Production and Pests Problems

Rice is intimately involved in the culture as well as the food ways and economy of many societies. It is an integral part of many peoples way of life and remains today as a leading crop and one of the most preferred foods around the world. It is certain that the domestication of rice ranks as one of the most important developments in history, for this grain has fed more people over a longer period of time than any other crop (Huke and Huke, 1990). There are two cultivated and twenty-one wild species of genus *Oryza.* The Asian cultivated rice *O. sativa* is grown all over the world whereas African cultivated rice, *O. glaberrima* is grown on a small scale in West Africa.

Ghana has relatively high rainfall and rice can be grown almost everywhere in the country. Rice schemes are found right on the border with Burkina Faso as in the case of Tono and down to the sea coast. Rice consumption in Ghana is estimated to be 80,000 tons of rice per year and currently the country produces sufficient to satisfy half of this demand MOFA (2008). Rice is estimated to contribute 15% of Agricultural gross domestic products (AGDP) of Ghana and covers 45% of land area planted to cereals (Kranjac-Berisavljevic, 2000). In Ghana rice is grown in three ecologies; upland rainfed, lowland rainfed and iriigation schemes (Seini and Asante, 1998). The Ghana irrigation development authority (GIDA) has 20 on-going irrigation projects scattered throughout the country. This covers

an estimated area of 10,000 hectares out of a potential area of 500,000 ha representing 2% of the country's arable land (Owusu *et al.,* 2001).

The introduction of irrigation schemes around the country has given farmers in these irrigation areas the opportunity to grow rice continuously throughout the year and farmers have over the years tended to adopt modern rice varieties in a bid to increase productivity. The photoperiod insensitivity and reduced growth duration of the modem varieties have made it possible to grow two and even three crops per year, where water, and temperatures are adequate. Continuous cropping throughout the year has caused shifts in the composition of pest fauna. Species dependent on standing water, such as the whorl maggot, *Hydrellia philippina,* and the rice caseworm *Nymphula depunctalis,* have become more abundant because of increased area under irrigation. Although rice insects have been a problem through the centuries, outbreaks have increased and the insect pest complex has changed in the last four decades. Some insects have increased in severity, whereas others have declined in importance.

Insects reduce yields substantially, especially in tropical areas. Insect pests attack all portions of the rice plant and all stages of plant growth. Feeding guilds consist of the root feeders, stem borers, leafhoppers and planthoppers, defoliators, and grain sucking insects. Insects also attack rice grains in storage. Major rice pests include the brown planthopper the rice gall midge, the rice bug, the rice leafroller, caseworms, rice weevils, stemborers, panicle rice mite and rats (IRRI, 2006).

2.2 Insecticides Use in Agriculture

Agriculture is a key industry with a central socioeconomic position in developing countries such as Ghana. According to statistics in the year 2000, Ghana's agricultural sector accounted for about 65% of the country's work force, about 40% of the gross domestic product, and about 40% of foreign currencies acquired through exports.

Globally, 95% of insecticides produced are used in agriculture (Overgaurd, 2006), and the consumption of insecticides in this industry is continuously increasing. Rice crop became a major insecticide consumer when traditional varieties were replaced by high yielding ones. These new varieties are more susceptible to pests and need to be protected by insecticide treatment (Sharma and Mchrotra, 1986).

Farmers over the years have adopted chemical control as the main tool used in controlling insect pests. This is because insecticides are relatively cheap, results are quickly obtained and application and handling requires less labour. Farmers especially those in developing countries lack adequate knowledge and appropriate equipments to ensure proper insecticide usage. According to a study in Northern Thailand, overall pesticide use was inappropriate, farmers did not wear suitable personal protection, apply pesticides in an appropriate fashion, or discard the waste safely. They frequently relied on commercial advertisements for the best pesticide to use (Plianbangchang *et al.,* 2009). In Ghana, studies by Ntow *et al.* (2006) showed serious lack of adequate knowledge in pesticide

handling and application among tomato farmers in Akumadan. Many instances of inappropriate practices in the handling and use of pesticides were observed and farmers did not necessarily associate hazardous pesticides use with better pest control.

2.3 Role of Agricultural Insecticides in Vector Resistance.

The use of insecticides in agriculture can exert a selection pressure on both the larval and adult stages of vectors. For example some of the mosquito breeding sites created by agricultural practices (irrigated rice fields) are sprayed directly when the crops are treated with insecticides and the insecticides can also drift as a results of wind or water to nearby mosquito breeding sites. When breeding sites are reached by agricultural treatments, all the mosquito larvae are subjected to selection pressure which is likely to induce resistance than the house spraying which reaches only anthropophilic females. In addition, insecticide residues in soil and water in mosquito breeding grounds also potentially contributes to resistance development. According to Muir (1982) insecticide residues in breeding sites could act as sub-lethal doses for genetic selection of resistance.

The use of insecticides to control insect pests of crops has been in many instances implicated in the rapid development of resistance by vectors breeding within or around the area. This can be attested to by the appearance of resistance in vector species prior to the use of chemical control for their specific control. Mouchet (1988) presented a case where disease vectors were found to be resistant to compounds of insecticide which had never

been used in public health but were employed in agriculture. High resistance has been observed in vector populations in agricultural areas sprayed than in population without such treatments. Diabate *et al.* (2002) reported that *Anopheles gambiae* s.l was resistant to permethrin and DDT in cotton growing areas but susceptible in areas of limited insecticide pressure (control areas). There are instances where there is positive correlation between the use of agricultural insecticide and resistance level in vectors. According to Brogdon *et al.* (1988) presence of the acetylcholinesterase and elevated esterase resistance mechanisms in *An. albimamus* was associated with intensely managed agricultural areas in Guatemala. Hemingway *et al.* (1986) also indicated that in Sri Lanka, agricultural insecticides were the source for selection pressure for resistance in *An. nigerrimus*. Georghiou (1982), recognized relevant points to implicate agricultural insecticides use in the development of vector resistance. These include among others temporary decrease or suppression of vector populations in areas sprayed for agricultural purposes without any public health use of insecticides. Normally these populations built up again when the vector species become resistant.

2.4. Adverse Effects of Pesticide Use in Agriculture

Use of chemical insecticides in agriculture has recorded many successes. It still remains a big weapon in the fight against insect pests of crops. A WHO (1986) estimate puts insecticide use in agriculture at 90% of total insecticide usage. In agricultural landscapes, rural and municipal residents can be exposed to agricultural pesticides either directly during crop applications or indirectly in air, water, or food. In the northern Great Plains of

the United States and Canada, pesticides have been detected in atmospheric samples, in surface and groundwaters, and in a variety of food products. Studies in the United States (Garry *et al.,* 1996), Spain (Garcfa-Rodriguez *et al.,* 1996), and New Zealand (Hanify *et al.,* 1981) have shown that environmental exposure to agricultural chemicals is associated with increases in human health anomalies. These include reduced stamina, gross and fine eye-hand coordination, and cognitive abilities in children (Guillette *et al.,* 1998), an increased incidence of human birth malformations (Garry *et al.,* 1996; Hanify *et al.,* 1981; Schreinemachers, 2003); and cryptorchidism in male children (Garcfa-Rodriguez *et al.,* 1996).

Indiscriminate use of chemicals in the control of various insect pests has been associated with some serious problems that affect man directly and indirectly. If improperly used insecticides can cause direct human poisoning, accumulate as residues in food and the environment or lead to the development of resistant strains of both target and non target pests. In Ghana, there are already some levels of contamination of pesticides in water, sediment, crops and human fluids in areas of highly intensive vegetable production (Ntow, 2001**).**

2.4.1 Contamination of the Environment (soil and water)

Insecticides may contaminate soils during application to crops, through direct application to the soil surface or incorporation in the top inches of soil during soil preparation activities. On the other hand insecticides can enter ground water resources through

percolation and surface run off during rainfall, thereby contributing to the risk of environmental contamination. The fate of pesticides in soil and water environments is influenced by the physico-chemical properties of the pesticide, properties of the soil and water systems (presence of clay materials, organic matter, pH), climate, biology and other factors (Gamilescu, 2005).

Pesticide residues in surface water and in soil are extremely important because of their potential impacts on aquatic ecosystems and their implications on drinking water sources (Ntow *et al,* 2005). Of particular importance are the organochlorines (OCs) insecticides, due to their high toxicity, their persistence in the physical environment and their ability to bioaccumulate in food chains (Jireies *et al.,* 2002). The OCs has been used in Ghana for over forty years, both for agricultural and public health purposes, with their residues having been detected in water, sediments, vegetable crops and in human fluids (Ntow *et al,* 2001). Despite the shift in preference for organochlorine and organophosphate insecticides to pyrethroids insecticides for agricultural purposes and the official limitation of OC insecticides, they are still available and in use in parts of Ghana (Ntow, 2005).

2.4.2 Pesticide Residues in Food

The establishment of maximum residue levels (MRLs) in foodstuffs is due mainly to the concerns of food safety expressed by people in the developed world. MRLs represent the maximum amount of residues that might be expected in/on a commodity during pesticide

use if good agricultural practices are applied. The public concern about food safety in the developed world has led to the establishment of maximum residue levels (MRLs), which restrict level of pesticide residues in foodstuffs. MRLs are established, taking into account the persistence of the particular pesticide in a given crop, the toxicity of the chemical and how much of the final product is typically eaten by the consumer (Chan, 2000).

According to PAN (1998) residues in food can arise from the use on a crop of legally allowed pesticides at a time interval too close to time of harvest, over- use of a of legally permitted pesticide, illegal use of pesticide that is not approved for that crop and incorrect use of pesticides for post-harvest treatment. In developing countries such as Ghana farmers are not equipped technically and lack the necessary technology to strictly adhere to MRLs in food crops. Therefore product of food crops in these countries normally contains high level of residues. Analysis of samples of street vendored food in Accra carried out in 1999-2000 revealed disturbing levels of contamination by heavy metals, pesticides, micro-organisms and mycotoxins (NRI, 2001). Chlopyrifos was detected in six out of eight samples of “Waakye” (rice and beans) and one out of eight samples of “Fufii” (cassava and plantain dough) (NRI, 2001). When MRLs are set, care is taken to ensure that maximum levels do not give rise to toxicological concerns (FAO/ WHO, 1993).

2.4.3 Effects on Human Health.

All pesticides must be considered potentially toxic to humans and animals. The hazard in the use of pesticide materials lies in failure to follow precautions and directions for use as indicated on the label or unexpected accidents such as the bursting of spray hoses, breakage or rusting of pesticide containers and careless storage or improper disposal of containers.

Pesticide poisoning occurs when chemicals intended to control a pest affect non-target organisms such as humans. Man may be exposed to pesticides through various routes such as ingestion (swallowing), inhalation (breathing), skin contact and eye contact. According to (Smith *et al.,* 2001) health problems caused by exposure to pesticides include acute poisoning and longterm effects on the individual's developmental, immunological, neurological and reproductive processes and it may as well cause carcinogenic effects. Most insecticides have been associated with different forms of cancer such as leukemia, Non hodgkin's lymphoma, prostate cancer and cancer of the ovary (Alavanja *et al.,* 2004). People most at risk are farmers or farm workers directly involved in handling and application of pesticides. Children are highly vulnerable to pesticide poisoning this is due to their high daily consumption of air, water and food per unit body weight and the immature detoxification system in the liver (Jurewicz *et al.,* 2006).

Exposure to some pesticides, particularly the organophosphates, destroys important enzymes in the nervous system. Repeated exposure may, without producing symptoms, progressively increase susceptibility to poisoning.

2.5 Socio-Economic Burden of Malaria

Where malaria prospers most, human societies have prospered least. The global distribution of per-capita gross domestic product shows a striking correlation between malaria and poverty, and malaria-endemic countries also have lower rates of economic growth. There are multiple channels by which malaria impedes development, including effects on fertility, population growth, saving and investment, worker productivity, absenteeism, premature mortality and medical costs (World Malaria Report, 2005).

Malaria continues to be an important vector-borne disease and a leading cause of morbidity and mortality in Africa South of the Sahara (RBM/WHO/UNICEF, 2005). Eighty per cent of global cases occur in tropical Africa, where the disease accounts for 10% to 30% of all hospital admissions and is responsible for 15% to 25% of all deaths of children under the age of five (WHO, 2008). Around 800,000 children under the age of five die from malaria every year, making this disease one of the major causes of infant and juvenile mortality. Pregnant women are also at risk since the disease is responsible for a substantia] number of miscarriages and low birth weight babies. The disease makes substantial demands on Africa's fragile health infrastructure, where the conventional treatment and control

strategies have proved ineffective (Morel *et al.,* 2003). Pregnant women and children below the age of five years are at a higher risk of infection (Akazili, 2002).

Malaria has social consequences and is a heavy burden on a country's development efforts. The disease is a major threat to the world's socio-economic development and is also a major health burdens in sub-Saharan Africa, where 15% of all disability life-years are lost to malaria (Chima *et al.,* 2003). It is estimated that a single bout of malaria costs a sum equivalent to over 10 working days in Africa. The cost of treatment is between $US0.08 and $US5.30 according to the type of drugs prescribed as determined by local drug resistance. In 1987, the total "cost" of malaria - health care, treatment, lost production, etc. was estimated to be SUS800 million for tropical Africa and this figure is currently estimated to be more than $US1,800 million (Akazili, 2002).

In Ghana, malaria is a major public health problem with its burden and transmission patterns varying across the country. Approximately 70% of the country is at risk of malaria infection and the disease accounts for 40% of all outpatients' attendance and 19% of all admissions in the health facilities (Ministry of Health, Ghana, 2006)

2.6 Malaria Vectors

The epidemiology of malaria in a given environment is the result of a complex interplay between man, *Plasmodia* and *Anopheline* mosquitoes. These three elements have to be

present for malaria transmission to occur in nature. The human organism is the natural shelter where the plasmodium parasites thrive, multiply and differentiate in sexual forms. It represent at the same time the favourite source of blood meal for the female of several *Anopheles* species and this allows the relationship among the three partners contributing to the malaria cycle to be established.

Malaria is transmitted between people through the bite of a female *Anopheles* mosquito infected with the *Plasmodium* parasite. More than 60 species have been incriminated in the transmission of infection (there are about 430 species of Anopheles, and about 3500 species of mosquito altogether).

Some species are more significant than others as vectors because of variations in susceptibility to the parasite or the propensity of the mosquito to bite humans and to enter houses when looking for a blood meal. *Anopheles gambiae* is the principal malaria vector in Africa and globally the most important vector of the disease. According to Depiney *et al* (2004), *Anopheles gambiae* s.s globally the most important vector is widely distributed in lowlands throughout tropical Africa. *Anopheles funestus* is also regarded as a very important vector in some areas in Africa especially when it is found to be associated with *Anopheles gambiae* s.l. Studies done by Appawu et *al* (2001) showed that *Anopheles gambiae* s.l and *Anopheles funestus* are the most widespread malaria vectors in Ghana. In some areas, especially in Central Africa two other important vectors of local importance are *Anopheles nili* and *Anopheles moucheti* (WHO, 2005). Other malaria vectors include

Anopheles melas and *Anopheles merus* found in the coastal regions of Western and Eastern Africa respectively.

2.7 Life Cycle of Anopheles Mosquitoes.

Adult *Anopheles* mosquitoes both male and female feed on nectar and damaged fruits. But only females feed on animal blood to provide proteins for their eggs. The adult mosquito survives for between one week and one month.

Females lay their eggs in batches of 70-100 on the surface of water at night. The type of water used for egg laying is indicative of the mosquito species and includes irrigation channels, a pool of water in a tree trunk, and sewage effluent. In tropical temperatures the eggs hatch after two to three days.

The larvae lie just below the surface of the water and feed on algae, and after 7-14 days turn into pupae during a five-minute process. The pupa is comma-shaped and is the least active stage of the Anopheles life cycle. After two to four days the pupa metamorphoses into an adult mosquito. The adults emerge during late evening and are able to fly within minutes.

Mosquitoes usually mate during flight. The male is attracted to the female by the tone of her wing beat, and has antennae that act as sound receptors. Once mated, the female

searches out a blood meal, following sensory cues such as host odour, carbon dioxide and convection currents. It then seeks out a resting place, which may be indoors or outdoors depending on the species. When the blood meal has been digested, the ovaries develop and the mature eggs are laid at night.

2.8 Vector Control Strategies

The global strategy for malaria control is focused on efforts to have effective drugs which can be readily used against the Plasmodium parasite and to employ vector control strategies that can effectively reduce vector population. Vector control methods are based on the principal target of a particular strategy and the chain of transmission most affected. According to Najera and Zaim (2002), methods of reducing human-vector contact, methods aimed at reducing vector density and methods aimed at reducing adult vector mortality are the main strategies used in the control of the malaria mosquito vector. Successful attempts at malaria control have exploited the weak link in the life cycle of plasmodium, represented by the fact that most *Anopheles* mosquitoes which have picked up the infecting stage of the parasite die of natural causes before the process of sporozoite production have been completed. Increasing this mosquito mortality rate through various methods reduces the number of infective mosquitoes almost to zero (Curtis and Townson, 1998).

Larval control is appropriate if the larvae are present in small number of discrete habitats. Oils may be applied on water surface to suffocate pupae and larvae and also render the environment unsuitable for *Anopheles* breeding. Biological insecticide such as the *Bacillus thuringiensis var. israeliensis* (Bti) can also be used for larval control. Bti is specific as it is formulated against mosquitoes, blackflies and midges. Walker and Lynch (2007) indicated that environmental management involving temporary or permanent removal of *Anopheline* habitats and larviciding with chemicals and biological agents can be helpful in controlling mosquito population.

At present, indoor residual spraying (IRS) and the use of insecticide treated bed nets (ITNs) are the main measures employed by WHO in the control of the mosquito vector. These control measures are often used in concert to reduce the number of infective bites to reduce transmission, coupled with prompt treatment of malaria cases by effective anti-malarial drugs (Coleman *et al.,* 2006).

The mosquito vector is versatile and is capable of adapting to control measures tailored against it over time. Therefore a well designed control strategy is required for proper and effective control. There is advocacy on the use of integrated vector management which includes the simultaneous combination of a number of control methods in which chemical control is used as a last resort. This is complicated by the fact that just a small population of mosquitoes would be enough to transmit the disease due to the high vectorial capacity

that is associated with mosquitoes. The appropriate use of a combination of methods can prove to be useful in bringing the vector population down and subsequently the incidence of malaria especially in Africa. For example, in Sri Lanka concentrated use of IRS in high transmission areas, good ITN distribution around the country and larviciding have created reduced malaria incidence to the lowest level since 1967 and there has been no report of a malaria epidemic since 1992 (World Malaria Report, 2005).

2.9 Insecticide Resistance

Insecticide resistance in malaria vectors is a growing concern in many countries and requires immediate attention because of the limited chemical arsenal available for vector control. Insecticide resistance can be defined as the ability of the majority of a population of insects to tolerate doses of an insecticide which will be lethal to the majority of individuals in a susceptible population. It is an inherited characteristic and hence can be transferred from one generation to another. According to Hemingway *et al.* (2004) insecticide resistance is a heritable characteristic involving changes in one or more insect gene.

Insecticide resistance is a genetic change in the ability of a population and it is reflected in the repeated failure of an insecticide to achieve the expected level of control. Resistance has been reported to develop to every class of insecticide, including 4th generation insecticides such as microbial products and insect growth regulators. Hemingway and

Ransom (2000) reported that many cases of resistance have been observed in mosquitoes due to the fact that they have short life cycles with abundant progeny, important features suited for early development of resistance. Despite decades of international efforts, a detailed practical description of insecticide resistance that would allow control strategies to be adjusted to specific needs remains the exception rather than the rule.

2.10. Resistance Mechanisms

Insecticide resistance mechanisms have a biochemical basis (Brogdon and McAllister, 1998). The two major forms of biochemical resistance are target-site resistance, which occurs when the insecticide no longer binds to its target, and detoxification enzyme-based resistance, which occurs when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferases (GST) prevent the insecticide from reaching its site of action. An additional mechanism based on thermal stress response has been proposed (Patil *et al.,* 1996) but its importance has not been assessed.

2.10.1 Target-Site Mechanisms

Alterations in the amino acids responsible for insecticide binding at its site of action cause the insecticide to be less effective or even ineffective. The target of organophosphorus (OPs) (e.g., malathion, fenitrothion) and carbamate (e.g., propoxur, sevin) insecticides is acetylcholinesterase (AcChE) in nerve synapses, and the target of DDT and synthetic pyrethroids are the sodium channels of the nerve sheath. DDT-pyrethroid cross-resistance

may be produced by single amino acid changes (one or both of two known sites) in the axonal sodium channel insecticide-binding site (Miyazaki *et al.,* 1996 and Williamson *et al.,* 1996). This cross-resistance appears to produce a shift in the sodium current activation curve and cause low sensitivity to pyrethroids (Vais *et al.,* 1997). Similarly, cyclodiene (dieldrin) resistance is conferred by single nucleotide changes within the same codon of a gene for a y-aminobutyric acid (GABA) receptor (Ffrench-Constant *et al.,* 1997).

2.10.1.1 Acetylchholinesterase (AcChE)

Organophosphates and carbamates insecticides affect AcChE which is a key enzyme in the nervous system. It enables nerve impulses to terminate by catalyzing the hydrolysis of the neurotransmitter acetylcholine (Vontas *et al.,* 2002). These insecticides (Organophsphate and Carbamate) function by effectively binding to the AcChE and rendering it ineffective. This leads to the build up of acetylcholine in the synapse causing the nerve to fire continuously eventually leading to paralysis and death.

At least five point mutations in the acetylcholinesterase insecticide-binding site have been identified that singly or in concert causes varying degrees of reduced sensitivity to organophosphates and carbamate insecticides (Mutero *et al.,* 1994). Resistance to organophosphate and carbamate insecticide based on reduced sensitivity of AcChE has been detected in *Anopheles gambiae* from Cote d' Ivoire (Weill *et al.,* 2003). Djogbenou *et al.* (2007) reported high levels of resistance to various carbamates and organophosphates in a resistance strain of *Anopheles gambiae* s.s in Burkina Faso. In Mozambique high AcChE

activities were found during a study on the efficacy of a number of insecticides even though vector control with carbamates (bendiocarb) was not negatively affected (Weill *et al.,* 2004).

2.10.1.2 Sodium-Potassium Ion Channels

Point mutations at the target sites of insecticides, decreasing the affinity of the insecticide to its receptor, constitute the second major and most widespread mechanism by which insects are able to resist insecticides (Brogdon *et al.,* 1998). Two mutations at amino acid position 1014 of the voltage-gated sodium channel, changing either a Leucine residue to a Phenylalanine (L1014F) (Martinez-Torress *et al.,* 1998) or a Leucine to a Serine (L1014S) (Ransom *et al.,* 2000) have been identified in *Anopheles gambiae* and confer knockdown resistance *(kdr)* to DDT and pyrethroid insecticides.

In West Africa *kdr* in *An. gambiae* has been associated with the single point mutation leading to a leucine-to-phenylalanine substitution (Martinez *et al.,* 1998) whereas in East African *An. gambiae* the leucine-to- serine substitution is prevalent (Ransom *et al.,* 2000). Both these mutations also confer cross-resistance to DDT due to its shared mode of action with the pyrethroids. The mutations are not specific to particular individuals since for example Verhaeghen *et al.* (2006) reported the presence of both types of mutation simultaneously in a population of *An. gambiae* s.s in Uganda. In a study conducted by Phillepe *et al.* (2009) in Cameroon increases in DDT and pyrethroid resistance, as observed in most areas, were generally associated with an increase in the relative

frequency of the S molecular form carrying the *kdr* mutations at higher frequencies. In South Western Chad, resistance to pyrethroids and DDT was associated with the L1014F *kdr* mutation in the S form of *An. gambiae* s.s. however, alternative mechanisms, probably of metabolic origin are involved in *An. arabiensis* (Kerah-Hinzoumbe *et al.,* 2009).

2.10.1.3 Gamma-Aminobutyric Acid (GABA) Receptors

The GABA receptors belong to the superfamily of neurotransmitter receptors that also includes the nicotinic acetylcholine receptors. These receptors are formed by the oligomerization of five subunits around a central transmitter- gated ion channel. An alanine-to-serine substitution in the putative channel-lining domain of the GABA receptor confers resistance to cyclodiene such as dieldren (Gamma HCH) (French-Constant, *et al.,* 1998). The mutation was first identified in Drosophila but has since been shown to occur in a broad range of dieldrin resistant insects, including *Aedes aegyptii* (Thompson *et al.,* 1993). The only variation in resistant insect is that glycine rather than serine can sometimes be the substituted amino acid residue. The insect GABA receptor is a site of action for pyrethroids and avermectin as well as cyclodienes and is the major inhibitory neurotransmitter in both insects and vertebrates. Interestingly, resistance seems to be able to persist in the absence of extensive insecticide selection, representing a threat for novel insecticides interacting with binding site for cyclodienes, such as fipronils (Ffrench-constant *et al.,* 2000).

2.10.2.0 Detoxification Mechanisms

The enzymes responsible for detoxification of xenobiotics in living organisms are transcribed by members of large multigene families of esterases, oxidases, and glutathione-s' transferases (GST). Perhaps the most common resistance mechanisms in insects are modified levels or activities of esterases that detoxify by hydrolyzing ester linkages in a wide range of insecticides.

2.10.2.1 Monoxygenases

The cytochrome P450s belong to a vast superfamily. Of the 62 families of P450s recognized in animals and plants, at least four (families 4, 6, 9, 18) have been isolated from insects. The insect P450 oxidases responsible for resistance have belonged to family 6, which, like the esterases, occur in Diptera as a cluster of genes (Maitra *et al,* 1996). Members of the cluster may be expressed as multiple (up to five) alleles (Tomita *et al.,* 1995). Enhanced levels of oxidases in resistant insects result from constitutive overexpression rather than amplification (Tomita and Scott, 1995). Reports of monoxygenase-based resistance are relatively rear in mosquitoes (Hemingway *et al.,* 1998). In East and West Africa some cases of pyrethroid resistance linked to increase levels of monoxygenase was reported by Brogdon and McAllister (1997). The cytochrome P450 oxidases (also termed oxygenases) metabolize insecticides through 0-, S-, and N-alkyl hydroxylation, aliphatic hydroxylation and epoxidation, aromatic hydroxylation, ester oxidation, and nitrogen and thioether oxidation (Wilkinson, 1976).

In *Diptera,* the esterases occur as a gene cluster on the same chromosome (Campbell *et al.,* 1997). Individual members of the gene cluster may be modified in instances of insecticide resistance, such as changing a single amino acid that converts the specificity of an esterase to an insecticide hydrolase (Newcomb *et al.,* 1997) or by existing as multiple-gene copies that are amplified in resistant insects (Mouches *et al.,* 1990 and Vaughan *et al.,* 1997).

2.11 Insecticide Resistance in Malaria Vector

The use of insecticides in malaria control programmes in Africa is expanding with the extensive and rapid roll out of long lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) (Roberts and Enserink, 2007). Twelve insecticides are approved by the World Health Organization (WHO) for IRS, but these belong to just four chemical classes (organochlorines, organophosphates, carbamates and pyrethroids) (Brown, 1958). All four of these classes are nerve poisons and either target acetylcholinesterase in the synapses or the voltage-gated sodium channel on the insect neurones. For insecticide-impregnated material, such as LLINs, the chemical arsenal is even more limited with only six insecticides, all from the pyrethroid class, available (Brown, 1958). These same insecticide classes are also widely used to control agricultural pests in Africa and this can pose additional selection pressure on mosquitoes when insecticide contaminated ground water permeates their larval habitats. This intensive exposure to insecticides has inevitably resulted in the evolution of insecticide resistance in the *Anopheles* mosquitoes the main malaria vector in Africa.

Resistance to the organochlorines DDT and the now obsolete dieldrin was first reported in African malaria vectors in the 1950s and 1960s [Hamon *et al.,* 1968 and Brown, 1958]. Pyrethroid resistance was detected in African malaria vectors in 1993 (Elissa *et al.,* 1993). Since then there have been published reports of pyrethroid resistant populations of *Anopheles gambiae* s.l. in countries from west, central, east and southern Africa (Ndjemai *et al.,* 2009 and Awolala *et al,* 2009) and also in *Anopheles funestus* in Ghana, Mozambique and South Africa (Hargreaves *et al,* 2000 and Corbel *et al.,* 2007). Recently, carbamate and organophosphate resistant populations of *An. gambiae* have been reported in West Africa (Corbel *et al.,* 2007).

2.12. Insecticide Resistance Management

Malaria is one of the main public health problems in Africa, causing more than one million deaths per year and placing a strong burden on developing African countries (WHO, 2005). Vector control remains an important component of malaria prevention. The two main methods of malarial vector control are indoor residual spraying (IRS) and insecticide-treated nets (ITNs). The choice of method depends not only on the epidemiological setting and the strategic objectives of vector control, but also on the feasibility and existence of an appropriate delivery structure. In most countries of sub-Saharan Africa, where malaria transmission is stable and infrastructures for large-scale IRS do not exist, ITNs are more cost-effective. Recently, the development of long-lasting insecticidal nets (LLINs), which resist loss of insecticide during washing and extend the residual efficacy of the insecticide, has addressed the technical and logistical constraints associated with re-impregnation of

insecticide on the nets. During the last decade, LLINs have become the predominant method of preventing malaria in many malaria-affected countries (Lengeler, 2004). More than eighty studies carried out around the world have shown the effectiveness of treated nets in reducing the incidence of malaria morbidity by 50 % (Lengeler, 2004).

Malaria is a major public health problem in Ghana. The strategy employed by the National Malaria Control Programme is based on effective use of insecticide treated bed nets among vulnerable groups, such as pregnant women and children under five years of age and proper case management through the use of recommended anti-malarial drugs.

There is renewed interest in the use of insecticides for malaria control because of the effectiveness of insecticide-treated materials that show promise in reducing malaria transmission and morbidity in The Gambia (Greenwood *et al.,* 1993) and in Ghana (Binka *et al.,* 1996). Insecticide-treated bed nets (ITN) have been used successfully in the Kassena-Nankana district of Ghana for over a decade now; first as the earliest experimental intervention trial followed by routine use among most community members. This led to the adoption of ITNs as national malaria control policy in support of the Roll Back Malaria (RBM) control programme.

The appearance of resistance in the malaria vector mosquitoes to the insecticides used for the treatment of bed nets in other areas of Ghana such as Accra (Ben-Mahmoud, 2008) and in nearby countries is however a cause of concern. This is because vector susceptibility is a basic requirement for the success of any control programme such as the RBM programme.

Due to all year round irrigated agricultural activity and ITN use in many areas such as Okyereko and Kassena-Nankana district, it is logical that vector resistance to the commonly used insecticides will develop with time. At present the insecticides used for treatment of mosquito nets are limited to the pyrethroids. Therefore *Anopheles* strains that become resistant to these pyrethroid products will subsequently create a problem for the vector control strategy currently employed. Even though the use of pyrethroids is still effective in many areas, intensification of resistance monitoring activities is still required to monitor any possible emergence. The findings in a study by Francis-Anto *et al.* (2009) showed that *An. gambiae* and *An. fiunestus,* the main malaria mosquito vectors in the Kassena-Nankana district (Appawu *et al*1994) are susceptible to the insecticides being used in the treatment of bed nets in the malaria control programme.

There is however, the need for continuous monitoring of the pyrethroids as the efficacy is not very high. Despite limited monitoring activities, resistances are already reported in a number of malaria vectors including some populations of *Anopheles gambiae* in Africa notably in West African countries, including Cote d'Ivoire (Chandre *et al.,* 1999), Burkina Faso (Diabate *et al.,* 2004 and Dabire *et al.,* 2008), Ghana (Yawson *et al.,* 2004), Nigeria (Awolola *et al.,* 2005), Mali (Tripet *et al.,* 2007), and Benin (Corbel *et al.,* 2007 and Djouaka *et al.,* 2008). This resistance is due to a target site modification (Hemingway *et al.,* 2004) and/or an increase in the ability of the mosquitoes to metabolize the insecticide (metabolic resistance).

Monitoring of insecticide resistance should be an integral component of the planning and evaluation of both agricultural pest and vector borne disease control programmes. Such monitoring should be standardized to ensure compatibility of data from different sources. The standard developed and recommended by WHO (2006) should be followed in monitoring and evaluating susceptibility levels. At the moment, African countries and international donors (UNICEF, IMF, WHO, etc.) are investing in the massive distribution of pyrethroid-treated nets to protect populations from malaria.

It is vital to study alternative possibilities that will allow the maintenance of the efficacy of this tool and manage insecticide resistance. The mixture of two insecticides with different modes of action has been shown to be a promising strategy for control of pyrethroid-resistant mosquitoes, as well. According to study conducted by Armel *et al.* (2009) in Benin, the combination of carbamate-treated PPW (of polypropylene mesh) and a pyrethroid-treated bed net was extremely effective in terms of mortality and inhibition of blood feeding of pyrethroid-resistant *An. gambiae.* It is also necessary to continue to look for other methods of vector control. In the short term, the use of new insecticides or even other types of chemicals, alone or in combination, can be effective. One good example is the combination of repellents and insecticides, which has been shown to be promising in areas where *Anopheles* mosquitoes are resistant to pyrethroids [Pennetier *et al.,* 2005 and Pennetier *et al.,* 2008).

2.13. Insecticide Residues in Soil and Water.

In agricultural landscapes, rural and municipal residents can be exposed to agricultural pesticides either directly during crop applications or indirectly in air, water, or food. In the northern Great Plains of the United States and Canada, pesticides have been detected in atmospheric samples, in surface and groundwater, and in a variety of food products. Studies in the United States (Garry *et al.,* 1996), Spain (Garcfa-Rodrfguez *et al.,* 1996), and New Zealand (Hanify *et al.,* 1981) have shown that environmental exposure to agricultural chemicals is associated with increases in human health anomalies. These include reduced stamina, gross and fine eye-hand coordination, and cognitive abilities in children (Guillette *et al.,* 1998); an increased incidence of human birth malformations (Garry *et al.,* 1996; Hanify *et al,.* 1981; Schreinemachers, 2003); and cryptorchidism in male children (Garda-Rodri'guez *et al.,* 1996).

Insecticide residues occur in very small quantities in the environment including soil, water and in biological materials. The residue level is normally analyzed through a series of important steps which includes, sampling, extraction, concentration of samples, clean-up and identification and quantification of the residue level.

2.13.1 Sampling

Sampling for residue analysis is the process through which a representative of the area under study is obtained and is used to determine the average level of residue in the study

material. Residue analysis sampling is done based on the nature and history of the pesticide containing material (NRI, 1994).

2.13.2. Extraction

This is conducted using a solvent such as hexane to help extract pesticide residue of interest from other component of the sample matrix. Hexane has been exclusively used for the extraction of chlorinated hydrocarbons and organophosphate insecticides (Matsumura, 1985). Insecticides are soluble in both polar and nonpolar solvents and less soluble in aqueous solutions (Hetzel, 2002). The extraction procedure varies depending on the type of material or matrix that is being worked on. For example the relatively polar solvents such as methanol are recommended for the extraction of fatty substances while for non-fatty samples and those having high to medium moisture contents, polar water miscible such as acetone is used (Matsumura, 1985 and Yeboah, 2001).

2.13.3 Concentration of Samples

Nitrogen gas or rotary evaporator is used to concentrate the cleaned up extract. The reason for concentration is to reduce the volume of the solvent carrying the pesticide residue without loosing residue, thereby concentrating the pesticide to a detectable level (Hetzel, **2002).**

2.13.4 Clean-up

This is the step where co-extractives are removed from the analytical sample. Clean-up Methods include: liquid-liquid partitioning, adsorbent column chromatography, permeation chromatography and solid phase extraction (SPE) (Olson, 1988; Yeboah, 2001; Hetzel, 2002; Aboagye, 2002). Solid phase extraction which is a common technique is based on other clean up techniques such as the adsorbent chromatography. The SPE packing material or cartridges retain the pesticide when the extract is passed through without co-extractives and then eluted with appropriate solvents (Aboagye, 2002).

2.13.5 Detection and Quantification of Residues

a) Chromatographic Methods

The use of chromatographic methods for routine analysis of pesticide residues in food products is most popular. The common equipments used include: gas chromatography (GC), high performance liquid chromatography (HPLC), thin layer chromatography (TLC) (Hetzel, 2002; Aboagye, 2002).

The multiple methods are preferred in order to include as many pesticides as possible in a single run. This makes the analysis of a large number of pesticides relatively cost-effective. GC methods have always been predominantly applied in the last decades due to the unsurpassed separation power of the capillary columns and the choice of various selective and/or sensitivity detectors (De Kok, 2002). According to Hetzel (2002) the GC detection

makes the use of very convenient equipments which includes: electron capture, flame ionization, flame photometric, nitrogen-phosphorus and thermal conductivity detectors.

In recent years, gas chromatography-mass spectrophotometry (GC-MS) has been introduced. This combination effectively allows automated identification (based on full spectra identification) and quantification. This has further improved the performance of GC- multiple residue methods, not only as the scope of one single method but also in the quality of the data produced (DE Kok, 2002).

Liquid chromatography methods have also gained popularity due to the introduction of more new polar and/ or thermally labile pesticides. N- methylcarbamates, Phenylureas and benzoylureas, using UV diodearray or fluorescence detection are the well known multiple residue methods that have been developed. Similar development trends seen in the last decade(s) with GC-MS seem to emerge now with LC-MS. LC-MS is nowadays the method of choice especially for single-residue analysis as the procedure is short since clean-up can be minimized or even omitted (De Kok, 2002).

Thin layer chromatography technique is based upon partitioning a pesticide between a solvent and a thin layer of adsorbent, which is usually silica or alumina that has been physically bonded to a glass or plastic plate. Samples are applied in a solvent as spots or bands at the edge of the plate and the plate is then placed in a tank containing a solvent.

The solvent migrates up the plate by capillary action, taking the pesticide with it and depositing it at a given distance from the edge of the plate. Following complete development, the plate is removed from the tank and spot or bands left by the migration of the solvent are detected using several techniques such as visualization under UV light. Other techniques employ reagents to produce colours resulting from chemical reaction that is specific for pesticide/ reagent combination. Amounts of pesticide can be determined semi-quantitatively by comparison with standards that are developed on the same plate as the unknowns (Olson, 1988).

b) Bioassay method

The separation of insecticide chemicals from any other group of toxins and contaminants can be specifically done using bioassay methods. Choice of test organisms is made based on the animal's pesticide sensitivity and the ease with which large numbers of them can be reared (Matsumura, 1985; Aboagye, 2002). According to Mclaughlin (1991) the instars of *Artemia salina* leach are suitable for detecting insecticide residues and the adult *Artemia* is also found to be sensitive to a broad range of compounds at concentration of 0.01 ppm in about twenty four hours.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Field Studies

3.1.1 Study Area

The study was conducted at Okyereko (5° 24' N- 5° 35' N to 0° 25'- 0 36W), Gomoa district in the Central Region of Ghana. It is located 65 km away from Accra, the Capital of Ghana (Figure 1). The area is under control irrigation where irrigation pumps are opened every day from 8.00 am to 6.00 pm to supply water through constructed channels to the rice fields. It is a gravity fed irrigation land with reservoirs and a pump station for supplementary irrigation with a developed land area of 81ha and actual irrigated area of 47 ha. The number of beneficiary farming families is 131 (Personal communication, Eghan, 2009, Okyereko Agric Office). The coastal savannah vegetation is characterized by tall grass interspersed with few trees and shrubs and receives an annual rainfall of 760-1000 mm. The mean daily temperature ranged from 26 to30 °C. It has an entomological inoculation rate of 81.9 infective bites/man/year (ib/m/yr) and a sporozoite rate of 0.2% (Okoye *et al.,* 2005).

Okyereko

Figure 1. Regional map of Ghana showing Okyereko in the Central Region and a district map of the Central Region showing the Gomoa district

3.1.2 Survey on Insecticide Use Patterns

A questionnaire was developed (appendix 111) to obtain needed information from rice farmers in the study area. Fifty (50) rice farm owners were identified from the village and interviewed. The questionnaire was designed into sections to cover the following areas:

- Personal Information
- Land tenure/Rice production
- Incidence of pests/Choice and source of insecticides
- Insecticide Application
- Knowledge of insecticide use
- Mosquito problems and malaria incidence.

3.1.3 Field Sampling of Mosquito Larvae and Pupae

Anopheles mosquitoes were collected as larvae and pupae from breeding sites within the rice fields and in pools of water around the rice farms (Figure 2). The larvae and pupae were collected using copper ladles and were transported into plastic containers that have holes in their lids for ventilation. The characteristic resting position just below the surface film was used mainly to identify *Anopheles* mosquitoes. *Anopheles* larvae normally position themselves horizontally on the water surface unlike other species which usually have an angular alignment on the water surface.

Figure 2. Flooded Rice field, an ideal larval breeding site.

3.1.4 Field Sampling of Water for Residue Analysis

Water samples were collected in 2.5 L Winchester bottles from the rice fields in duplicates. The samples were taken from three different fields or spots where mosquito larvae were obtained. The bottles were sealed, labelled and transported to the laboratory where all the samples were filtered with Bucher filtration system using a vacuum pump. One litre of the water filtrate of each sample was concentrated to 100 ml at 40 °C using Buchi Rotavapour

(Buchi, Switzerland). The concentrates were transferred into 200 ml Duran bottles and stored at 4°C for further studies.

3.1.5. Field Sampling of Soil for Residue Analysis

Soil samples were collected in duplicates at a depth of 0-15 cm from the soil surface. Samples were taken from the spots where water samples were collected. The soils were collected into bottles wrapped with aluminum foil, labelled and transported to the laboratory. They were hot-air oven-dried at 40 °C for 72 hours and ground in a mortar into finer particles and sieved with a 1 mm sieve. The samples were mixed homogenously and stored at 4 °C for further studies.

3.2. Laboratory Studies

3.2.1. Laboratory Rearing of Mosquitoes

The larvae and pupae collected from the field were initially separated in the laboratory. The larvae were transferred into white plastic trays (5 cm high, 27 cm wide and 36 cm long) with a wide surface and water depth of about 2 cm (Figure 3). The samples are cleansed of all foreign organisms using a pipette. Ordinary tap water was used to replace the water from which the larvae and pupae were originally collected. Larvae were fed daily on a diet of finely ground gold fish meal (Nutrafin, Rolf, Hagen, USA). An adequate amount was added each time larvae were fed. Larval water was changed as and when it became too cloudy, in order to avoid contamination and to ensure normal larvae

development. Dark, comma shaped pupae were removed regularly by means of a Pasteur pipette and put into a small plastic beaker with water before being transferred into the rearing cages for adults to emerge (Figure 4). Adults were fed with 10% sugar solution that was soaked in cotton wool within 12 hours of post emergence. Non-blood fed 2-5 days old adult were used for bioassays.

During the rearing period, temperature was maintained at 25-33 °C, relative humidity at 55-80% and a photoperiod of 12 hours light and 12 hours darkness. Precautions were taken to prevent overcrowding of larvae in trays, and dead adults were removed regularly to avoid ant attraction and mould formation.

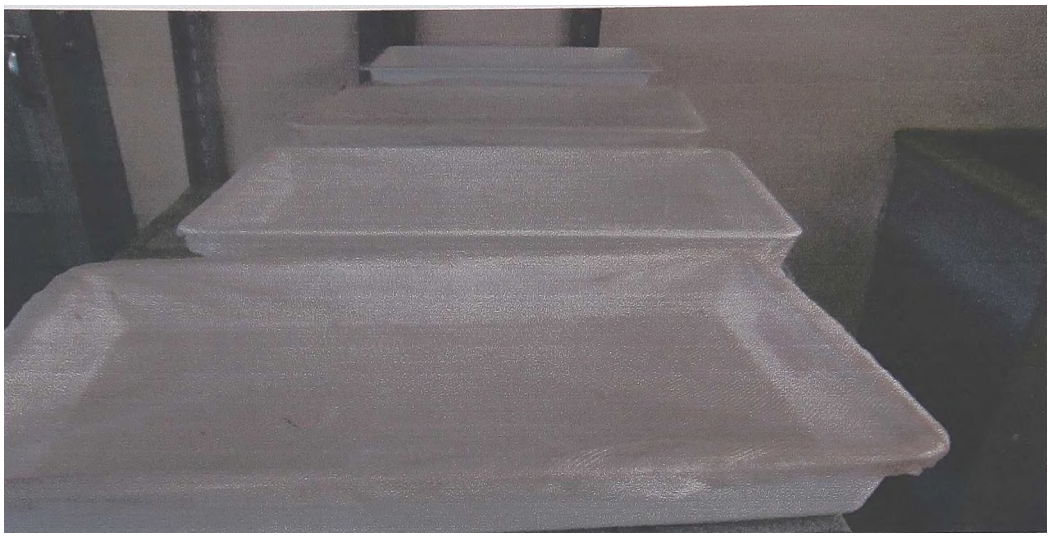

Figure 3. Trays used for rearing larvae

Figure 4. Cages used for holding pupae and adult mosquitoes

3.2.2 Susceptibility Tests

The WHO susceptibility tests kit (WHO, 1998) was used to carry out the bioassay for the wild and susceptible populations. Adult female *Anopheles* mosquitoes aged 2-5 days and non blood fed were used for the study. The mosquitoes were gently transferred from the cages into holding paper cups using an aspirator. Twenty mosquitoes were held in each cup for one hour and the weak and damaged ones were removed. Healthy-looking and fit mosquitoes were introduced into tubes lined with insecticide impregnated papers in batches of at most 10 at a time. The papers were impregnated with diagnostic concentrations of 5% malathion, 0.01% propoxur, 4% DDT, 0.75% permethrin and 0.05% deltamethrin. A control, which constituted paper impregnated with Dow coming 556 silicon fluid was set up for each test. The mosquitoes were exposed for 60 minutes and the number of mosquitoes knocked down was recorded at 5, 10, 15, 20, 30, 40, 50 and 60 minutes. Whenever knockdown at 60 minutes was less than 80%, an additional 20 minutes was allowed and knockdown recorded at 80 minutes. A mosquito was considered knockdown when it lay on its side on the floor of the exposure tube and was unable to fly (WHO, 1998).

The mosquitoes were transferred into tubes by gently blowing them through the open space between the exposure and the holding tubes. Mosquitoes were fed on 10% sugar solution in order to ensure that mortality was through the bioassay and not through starvation. Mortality was recorded 24 hours post exposure and each test was replicated four times together with a control. The resistance or susceptibility status was evaluated based on the WHO criterion: 98-100% mortality indicates susceptibility, 80-90% mortality indicates

need for confirmation and < 80% mortality suggests resistance (WHO, 1981; WHO, 1998). Abbott's formula (Abbott, 1925) was used to correct for mortality whenever mortalities in the control was between 5-20% and the experiment was repeated when mortality in the control exceed 20%. The bioassay results for the wild population were compared to that of the susceptible 'Kisumu' strain.

The survivors of each bioassay were immobilized and together with the dead mosquitoes transferred into labelled Eppendorf tubes perforated at the tip and put in Zip lock bags containing silica gel and stored until ready for use in the morphological identification and for molecular studies.

3.2.3. Morphological Identification of *Anopheles gambiae* s.l

Identification keys (Gillies and de Mellion, 1968) were used to morphologically identify *Anopheles* mosquitoes. The larvae of *Anopheles* lack siphon and lie parallel to the water surface. The pupae have short respiratory trumpets that are broad distally giving a conical appearance. The pupae also have short peg-like spines situated laterally near the distal margins of the abdominal segments two to seven (Service, 1980). The adults usually rest at an angle of about 45° 'to the surface and most have spotted wings. The females have non-plumose antennae and palps as long as the proboscis and usually lie closely alongside each other (Service, 1980) while the males have plumose antennae.

Anopheles gambiae s.l was also morphologically distinguished from *Anopheles funestus.* Whereas *Anopheles funestus* and other anopheles species have four pale spots on the coastal margin of the wings, entirely dark anal vein colouration and entirely dark tibia ornamentation, *Anopheles gambiae* complex possess five pale spots on the coastal margin of the wings, anal vein colouration with three white spots and a dark apical fringe and white speckle (spots in the median part) tibia ornamentation (Gillies and de Mellion, 1968).

3.3.1. Molecular Studies

Molecular techniques were used to identify *An. gambiae* s.l sibling species and the molecular forms of *An. gambiae* s.s. The presence and frequency of *kdr* mutation gene in the wild population was also analyzed. The reagents and solutions used are indicated in Appendix 1

3.3.1.1. DNA Extraction

Extraction of the DNA from adult *An. gambiae* s.l was done using the protocol described by Collins *et al.* (1987). Individual mosquitoes were homogenized in 100 p.l Bender buffer in a 1.5ml Eppendorf tubes followed by incubation at 65 °C for 30 minutes. Fifteen microlitres (15 (xl) of pre-chilled 8M potassium acetate was added to the homogenate and mixed well by tapping tube. This was followed by incubation on ice for 45 minutes. The solution was centrifuged at 14,000 rpm for 10 minutes using 5412C Eppendorf microfuge (Hamburg, Germany). The supernatant was transferred into a fresh tube and 2X (double the volume) volume of absolute ethanol was added, mixed well and was left to stand at

room temperature for 5 minutes. After that it was centrifuged at 14,000 rpm for 10 minutes. The ethanol was poured off and 100 (al of TE was added to redissolve the pellet before it was incubated at room temperature for 30 minutes. An aliquot of 5 p.1 of 5M NaCl and 210 |il of absolute ethanol was added followed by incubation at -40 °C for one hour. This was then spun down in the microfuge for 10 minutes at 14000 rpm and the ethanol was poured off before the pellet was inverted on a tissue paper to dry. The DNA pellet was redissolved in 25 |il TE RNase (5 |j.l/ml), and stored at -20 °C until ready for use.

3.3.I.2. PCR Identification of Members of *An. gambiae* Species Complex.

Anopheles gambiae sibling species identification was carried out according to the method of Scott *et al.* (1993). Five sets of primers abbreviated as UN, GA, ME, AR, and QD (Table 1) designed from the DNA sequence of the intergenic spacer region of *An. gambiae* complex of ribosomal DNA (rDNA) were used for species identification. The UN primer anneals to the same position on the rDNA sequences of all five species, GA anneals specifically to *An. gambiae* s.s., ME anneals to both *An. merus* and *An. melas,* AR to *An. arabiensis* and QD to *An. quadriannulatus.*

Table 1. Oligonucleotide primer sequences, melting temperatures and the expected band sizes of the PCR amplified DNA products for the identification of *An. gambiae* species complex.

Primer	**Sequence (5'- 3')**	**Band size(bp)**	**T_m(°C)**
UN	GTG TGC CCC TTC CTC GAT GT	468	56
GA	CTC GTT TGG TCG GCA CGT TT	390	62
ME	TGA CCA ACC CAC TCC CTT GA	464	90
AR	AAG TGT CCT TCT CCA TCC TA	315	78
QD	CAG ACC AAG ATG GTT AGT AT	153	54

Scott *et al.* (1993)

The PCR reaction mixture of 20 jj.1 contained lx reaction buffer (Buffer C), 200 |aM each of the four oligonucleotide triphosphate (dNTPs), 0.25 |*M each of the oligonucleotide primers and 0.5 U of DNA Taq polymerase enzymes. Two microlitres (2 (0.1) of mosquito DNA (from Bender buffer extraction method) template was used for the amplification reaction. The reaction mixture was topped up to 20 *il* with sterile double distilled water.

The reaction mixture was centrifuged briefly and overlaid with mineral oil to avoid evaporation followed by refluxing during thermo cycling. The PCR thermal cycling profile was as follows: an initial denaturation step of 3 minutes at 94 °C followed by 35 cycles

with denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds and extension at 72 °C for 60 seconds and ended with a final cycle of 94 °C for 30 seconds, annealing at 50 °C for 30 seconds and extension at 72 °C for 10 minutes using a PCR Express Thermal Cycle (HYbaid Ltd. UK).

The amplified products were analyzed by gel electrophoresis. Five (5 jj,I) of each PCR product was mixed with 1 (Jil of lOx bromophenol loading dye and electrophoresed in *2%* agarose gel stained with 0.5 ng/ml of ethidium bromide. The electrophoresis was run on lx Tris acetate- EDTA (TAE) buffer at 100 V for 30 minutes and visualized and photographed over a UVP dual intensity transilluminator at short wavelength using a Polaroid direct screen instant camera fitted with an orange filter, a hood and a Polaroid type 667 film. The film was processed as recommended by the manufacturer (Polaroid Inc, USA). The identity of a sibling species was established by visual comparison with its DNA size with the mobility of a standard 100 bp DNA ladder (Sigma, USA).

3.3.1.3 Identification of the Molecular Forms of ***Anopheles gambiae*** s.s

Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method was used to identify *An. gambiae* s.s molecular forms. The method of Fanello *et al. (2002)* involving a combination of the protocol established by Scott *et al.* (1993) and Favio *et al.* (1997) was used. This method allows for simultaneous identififcation of all species of *An. gambiae* complex as well as the M and S forms within the *An. gambiae* s.s. It is based on the fact that GCG^{A} C restriction site for Hhal enzyme (Favio *et al.,* 1997) lies

within the *An gambiae* specific fragment (Scott *et al.,* 1993) which makes it possible to digest this fragment directly in order to differentiate M and S molecular forms. This restriction site for Hhal (Fanello *et al,* 2002) is located at position 469 in all taxa except *An. merus* and of a second restriction site at position 475 in *An. quadriannulatus, An. melas* and *An. merus.* The *An. gambiae* S-form digestion is characterized by two fragments, 257 and 110 bp long, which results from the presence of the Hhal restriction site. The *An. gambiae* M-form does not have this restriction site and thus is characterized by a single 367 bp fragment.

The PCR reaction is the same as described in section 3.3.1.2. After amplification, 1U of Hhal enzyme (Promega, USA) in lOx enzyme buffer and nuclease free water were added to 10 (4.1 of PCR product to make a 20 \i\ reaction mix. The digestion was carried out at 37 °C for 6 hours in a thermal cycler and the products electrophoresed through ethidium bromide stained 2% agarose gel visualized under UV light. The *An. gambiae* S form digestion is characterized by two fragments, 257 and 110 bp long, which are due to the presence of Hhal restriction site. The *An. gambiae* M form does not have this restriction site and thus is characterized by a single 367 bp fragment.

3.3.I.4. PCR Detection of the kdr Alleles in Anopheles gambiae Complex.

The method of Martinez-Torres et al. (1998) was used to detect kdr alleles in the mosquitoes. The DNA extraction was performed as described in section 3.3.1.1. The primers used were Agdl and Agd2 (Oligos Etc. Inc., USA) and Agd3 and Agd4 (Owsel,

UK) [Table 2]. A total of twenty individual An. gambiae s.s mosquitoes were chosen at random for the kdr-PCR.

The *kdr* genotyping of individuals was possible after amplifying the DNA template from mosquitoes following the PCR conditions of 94 °C for 3 minutes (initial denaturation), followed by 45 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for one minute. There was a final cycle of 94 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for 10 minutes followed by cooling at 4 °C.

Table 2. Sequence details of the *kdr* primers and their melting temperatures (T_m).

Primer	Sequence (5'-3')	T_m (oC)
Agdl	ATA GAT TCC CCG ACC ATG	54
Agd2	AGA CAA GGA TGA TGA ACC	64
Agd3	AAT TTG CAT TAC TTA CGA CA	40
Agd4	CTG TAG TGA TAG GAA ATT TA	52

Martinez-Torres *et al.* (1998)

The products were electrophoresed through ethidium bromide-stained *2%* agarose gel and visualized under UV light. The *kdr* genotype of both the susceptible and resistant

individuals was then recorded. Expected sizes for susceptible, resistant and control are 137 bp, 195 bp and 293 bp respectively.

3.4.1 Isolation of Insecticide Residues from Soil and Water

Insecticides selected for this study were based on the results of the survey on insecticide use patterns conducted among rice farmers in the area. The respondents obtained from the survey shows that all the farmers use insecticides in controlling insect pests of rice such as the stem borers and caseworms. Lamdacyhalothrin and permethrin which are all pyrethroids were the main insecticides used by farmers in the area in recent years.

3.4.I.I. Residues Extraction from Soil and Water samples.

Standard protocols were used in extracting insecticide residues from soil and water samples.

3.4.1.2 Soil

Residues extraction from soils was done using the method of Singh *et al.* (1999) with slight modification. Hot-air dried soil (10 g) were put in a 100 ml conical flask and mixed with 25 ml hexane and ethyl acetate solution in the ratio 9:1. These were then stirred on a magnetic stirrer for 12 hours and the organic phase was decanted into a 100 ml flask. This process was repeated with fresh solvents. The two fractions were pooled together and centrifuged for 5 minutes at 3000 rpm using Gallenkamp bench centrifuge (radius of rotor = 11.5 cm). The supernatant was transferred into a clean 100 ml flask. Duplicate extraction was performed for each sample.

3.4.1.3. Water

Handa *et al.* (1999) method of extraction was used with slight modification. Fifty millilitres of the water concentrate was taken into a separating funnel in duplicates and shaken with 50 ml of hexane: ethyl acetate mixture (9:1) for 2-3 minutes. The organic phase was carefully transferred into clean flasks. The process was repeated thrice with fresh solvents and all three extracts were pooled and transferred into clean 250 ml flask containing anhydrous sodium sulphate (5 g) to remove moisture present.

3.4.1.4. Concentration of Extracts

Extracts were concentrated to about 5 ml using Buchi Rotavapour (Buchi, Switzerland) at 40 °C. They were transferred into pre-weighed vials and then evaporated under a stream of nitrogen gas to dry. The dry extracts were redissolved in 0.5 ml of hexane and stored at 4 °C for further use.

3.4.1.5. Clean up of Soil and Water Extracts

Methanol, ethyl acetate and hexane as eluting solvents were used to clean-up the extracts through C-18 solid phase extraction (SPE) columns. The C-18 cartridge SPE columns were pre-conditioned with 2 ml of methanol. The extracts (0.5 ml each) were applied to the top of the tube using a pipette. Two millilitres of eluting solvents in the order, ethyl acetate and hexane were passed through the columns to elute the sample. The effluents were collected into pre-weighed 5 ml vials and evaporated to dryness under a stream of nitrogen gas. The weight of the fractions recovered after the clean-up was determined and the extract was

reconstituted in appropriate volume of solvent to obtain a 10 mg/ml stock solution from which 1 mg/ml solutions were prepared for GC analysis.

3.4.I.6. Analysis of Insecticide Residues Using Gas Chromatography (GC) Method.

A Varian CP3800 Gas chromatograph (GC), was used to detect and quantify the residues in water and soil samples at the Chemistry Section of the Ghana Standard Board. The samples (1 JJ.1 each) were injected into the column of the GC with autosampler at a temperature of 225 °C. The compounds were partitioned through the stationary phase, a capillary column (30 m + 10 m EZ Guard, i.d. 0.25 mm, fused with silica coating VF-5 ms, 0.25 jim, film) carried by the solvents. The oven temperature was programmed at 90 °C (1 min) then 30 °C/min till 240 °C followed by 5 °C/min up to 300 °C for 3 min. The carrier gas was nitrogen maintained at a constant flow rate of 1 ml/min. The molecules of the insecticide residues were differentiated at different rates through the gas. The electron capture detector (ECD) was used to detect the compounds (usually the halogens) present. The detection temperature was 300 °C. The ECD was linked to a computerized integrated system which counts and records the signals as peaks, which were used to quantify insecticide residues present.

3.5 Data Analysis

The data from the insecticide use pattern survey was analyzed using SPSS 13.0. The values for the knock down times KDT_{50} and KDT_{95} were estimated from the time-mortality

regression using SPSS 13.0 probit analysis (Finney, 1971). Abbott's formula (Abbott, 1925) was used to correct for observed natural mortalities in the adult susceptibility tests.

CHAPTER FOUR

RESULTS

4.1. Survey on Insecticide Use Pattern

4.1.1 Demographic Information

A total of fifty (50) farmers were identified and interviewed for the survey on insecticide use pattern. There were more male (56%) than female farmers (44%) (Figure 5). The results showed that majority (56%) of the farmers were within the age range 41-50 years with 22% more than 50 years old while 18% fell within the range 31-40 years and only 4% of the farmers were less than 30 years (Figure 6). Almost forty percent (38%) of fanners interviewed did not attend formal school, with 30% of respondents at least having reached junior secondary level. A relatively small number (12%) of the farmers have attained secondary and tertiary level of education while 8% of respondents stopped going to school at the primary education level (Figure 7). All the farmers interviewed were small scale farmers. The rice farm land ownership scheme in the area is mainly leasehold and farmers do not rotate rice with other crops.

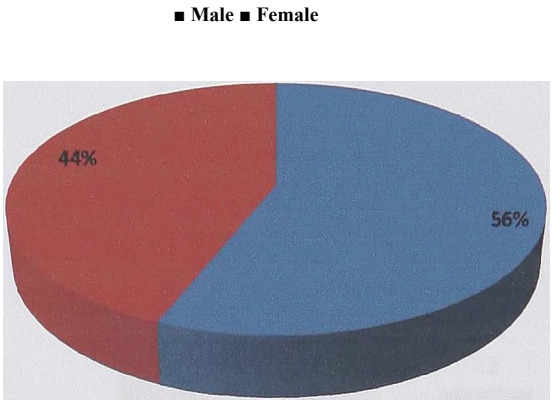

Figure 5. Percentage distribution of farmers by gender.

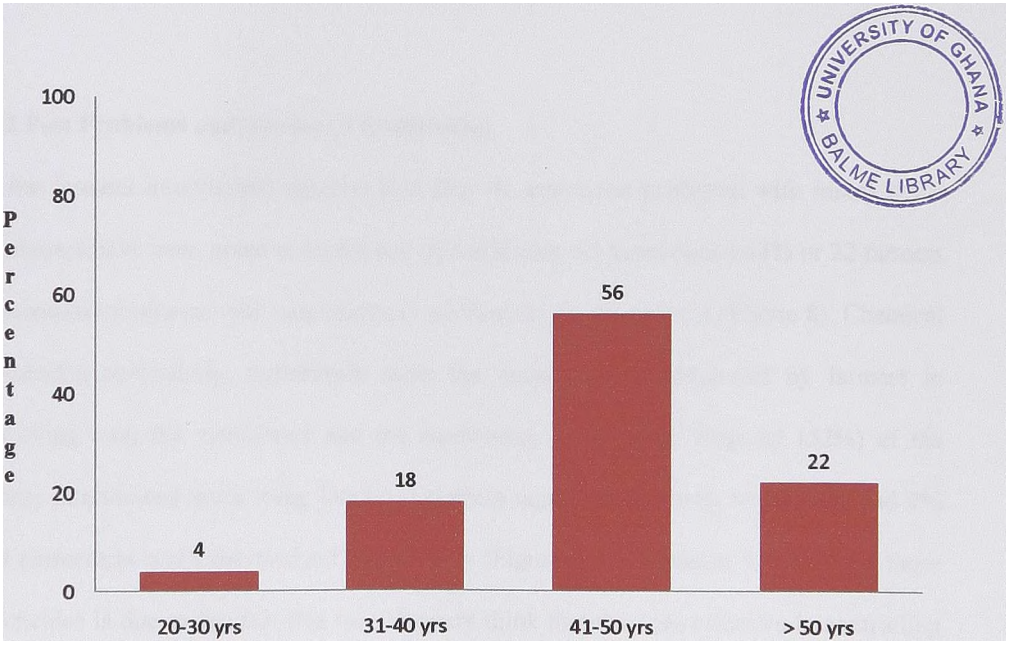

Figure 6. Percentage distribution of farmers within different age group.

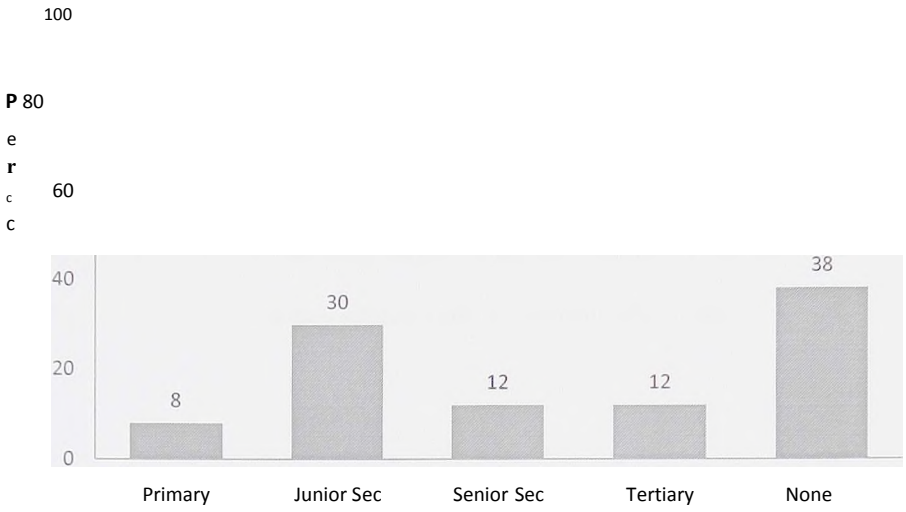

Figure 7. Percentage distribution of farmers based on level of educational.

4.1.2 Pest Problems and Choice of Insecticides

All the farmers interviewed reported that they do encounter problems with insect pests. Stemborers have been noted to be the key pest affecting all farmers and 44% or 22 farmers do encounter problems with caseworms in addition to the stemborers (Figure 8). Chemical insecticides particularly, pyrethroids were the main weapon employed by farmers in controlling both the stemborers and the caseworms in the area. Majority (52%) of the farmers interviewed were using lamdacyhalothrin against both pests, while 44% and 4% used permethrin and cypermethrin respectively (Figure 9). The reason for choosing these insecticides is due to the fact that most farmers think that they are effective in controlling the pests. However, few farmers also indicated that they normally use these insecticides because they are relatively cheap.

Insecticides are purchased after appearance of pest according to most (70%) farmers but a reasonable number (30%) of farmer usually buy their chemicals before the beginning of the growing season. Insecticides are normally purchased from chemical dealers and only few (18%) farmers obtain their insecticides from extension workers. According to the respondents, insecticides are normally stored in the farm house before and after use but about 30% of the farmers said they usually keep their chemicals at home.

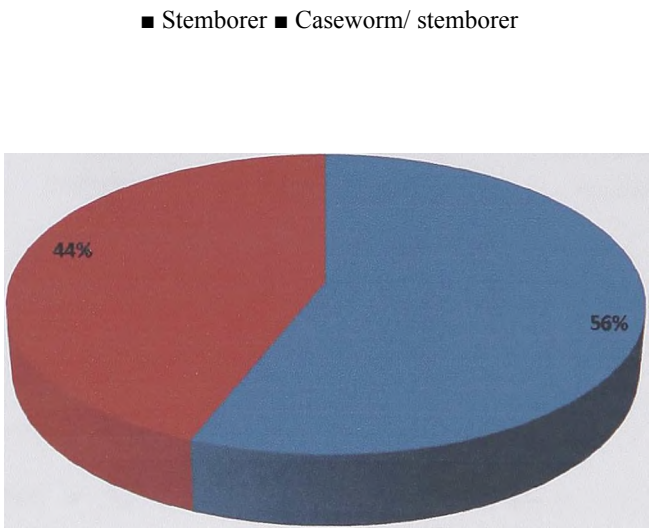

Figure 8. Percentage distribution of different pest observed on farms.

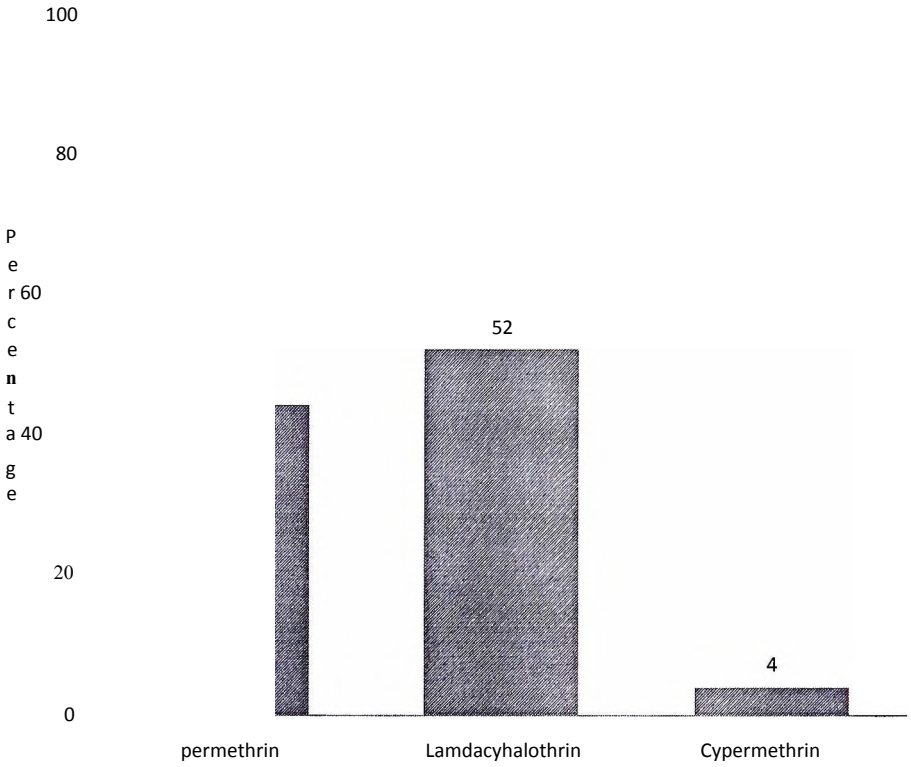

Figure 9. Percentage distribution of insecticides used by farmers.

4.1.3 Insecticide Application and Protective Clothing Used

Nursery application of insecticides was done by all the farmers interviewed. They generally used knapsack during application. However, some (30%) of the respondent applied insecticides using broom, cups or sometimes using the hand during nursery application. On the other hand, almost all the farmers in the survey use knapsack when applying insecticides in the field.

Only about 46% of respondents use protective clothing during field application of insecticides. Type of protective gears that farmers used during application of insecticides include gloves (40%), overall (34%), Wellington boots (30%), respirator (26%) and majority of the farmers used long sleeve shirts (80%), and long trousers (52%) (Table 3). Only 24% of the farmers interviewed consult extension workers when formulating their insecticides while the majority (76%) follows instruction labels of insecticide containers. Most farmers use a concentration of 21 and 25 ml/L when formulating their insecticides. While slightly less than half the number of respondents (46%) disposed off empty containers through burning them, the rest (54%) indicated they destroy them after use.

Table 3. Protective gears used during insecticide application.

Protective gears	**Percentage of respondent using gear**
Long sleeve shirt	80
Long trouser	52
Overall	34
Wellington boot	30
Gloves	40
Hat	48
Respirator	26
Goggles	3

4.1.4. Information and Technical Training on Insecticide Usage

According to most respondents information on insecticide use was normally obtained from the agricultural extension worker in the area. However, about 32% acquire information from chemical dealers while 4% obtained information from other farmers or from labels of

insecticide containers (Figure 10). Technical training which includes insecticide use is conducted by the agricultural extension officer and sometimes by officials from NGOs in the area and most farmers (84%) attended these training sessions. However, these training sessions took place only once in the year according to 66% of the farmers interviewed. Advice on insecticide use was obtained from the extension workers mainly but a reasonable number (26%) do obtain advice from commercial chemical dealers, whilst about 22% get advice from NGO officials.

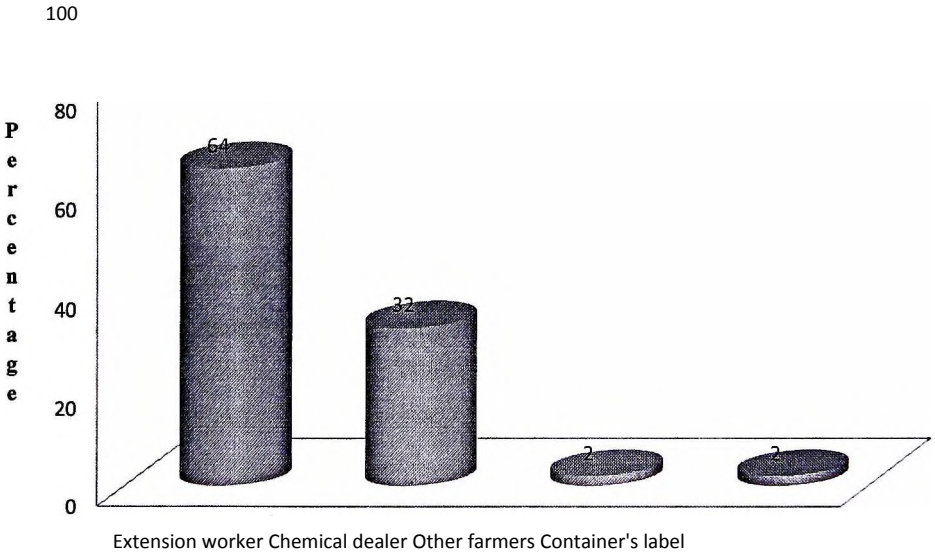

Figure 10. Percentage distribution of farmers based on information source on insecticide use

A large number of farmers (44%) look for advice mainly, on the type of insecticide to apply. However, some farmers (36%) were more interested on the frequency of insecticide application, and only 14% and 6% were interested in dosage of insecticide to be applied and safety in using insecticide respectively (Figure 11). While 78% of the respondents applied insecticides in the field after appearance of pests, 20% of farmers had spraying scheduled (calendar spray) which was strictly followed (Figure 12). About 66% of the respondents reported not encountering health problems after using insecticides but 44% indicated that they do encounter problems with insecticides. These problems include headache, diarrhoea (poisoning) and pollution of water bodies around the farm area.

More than half (51%) of the farmers interviewed observed a pre-harvest interval, after the last insecticide application normally of 1-2 weeks interval. However, 38% of the farmers in the study did not observe pre-harvest interval. Majority (70%) of farmers interviewed kept farm records on insecticide use in order to know when to apply insecticides again, and for future references.

Figure 11. Percentage distribution of farmers based on usefulness of advice given on insecticides

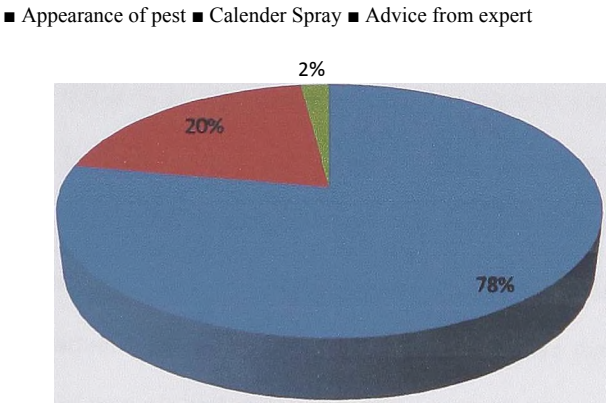

Figure 12. Percentage distribution of farmers based on timing of decision on insecticide application.

4.1.5 Mosquito and Malaria Problems

Mosquito related problems such as malaria were reported by all the farmers who participated in the survey. Mosquitoes are generally controlled in the area using insecticide-treated bed nets (ITN) but about 24% of those interviewed used aerosol spray, while 14% used mosquito coil (Figure 13). The main reason for employing these strategies was that they found them to be very effective. Small number (14%) of the respondent also reported using mosquito coils because it is relatively cheaper and readily available. All the respondents reported that they and their family members suffered from malaria at least once every year while more than 20% indicated that they frequently suffered from the disease (Figure 14).

The preferred mode of treatment for most farmers in the area was doctor's prescription but 20 and 22% of the respondent sought treatment from pharmacies and through the use of herbal practitioners respectively. The cost of malaria treatment per person ranged from five Ghana cedis (GH0 5) to forty Ghana cedis (GH0 40) with about 50% of the respondents paying GH0 5-10 (Five to ten Ghana cedis) (Figure 15). Some farmers reported changing insecticide brands and according to them this is due to differences in cost and in some cases due to mosquito resistance against certain brands of aerosols or coils used. More than half of the farmers interviewed think that mosquitoes are developing resistance against most brands of aerosols and coils used and according to them this is because mosquitoes do not die when certain brands of aerosols or coils are used against them. Finally all the farmers that participated in the study have some form of water body within or around their farms and they always observed mosquitoes breeding there.

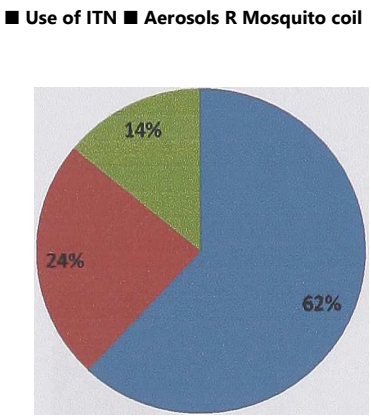

Figure 13. Percentage distribution of farmers based on control measures taken against mosquitoes.

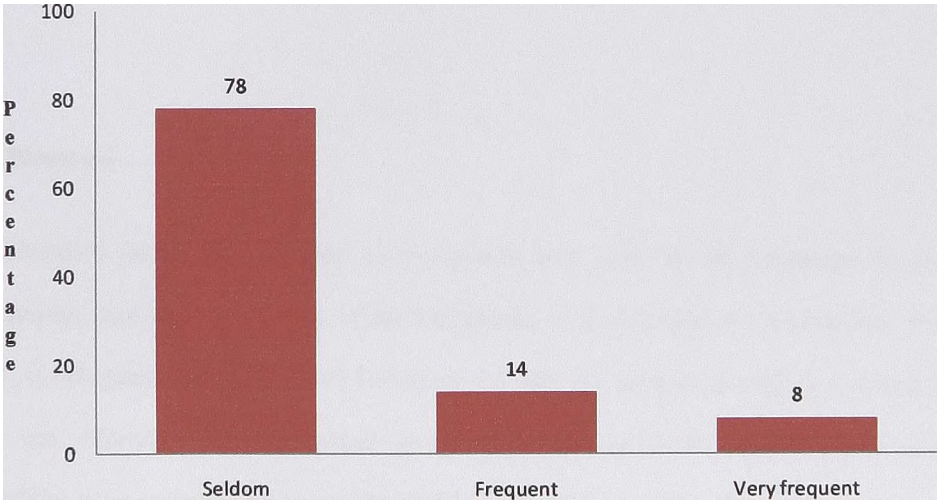

Figure 14. Percentage distribution of farmers based on frequency of incidence of malaria in their families.

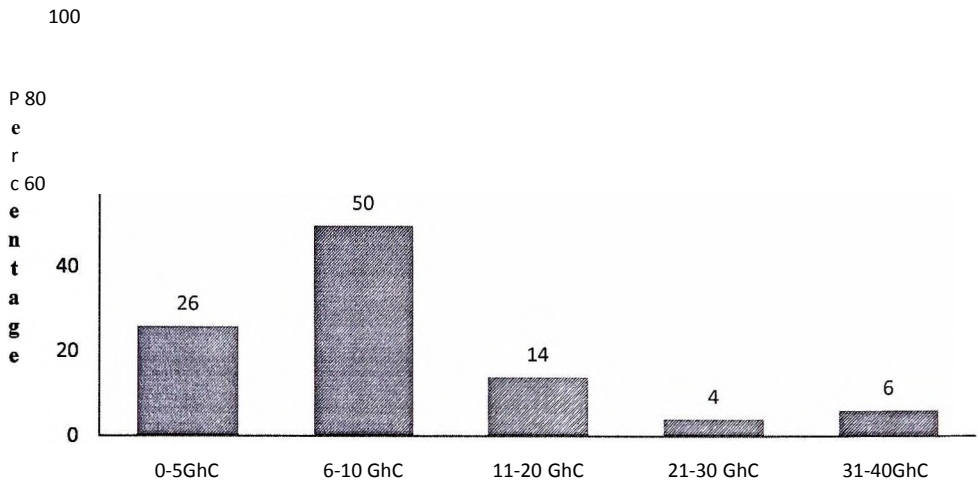

Figure 15. Percentage distribution of farmers based on expenditure on malaria treatment.

4.2. Bioassays

Five hundred female *An. gambiae* s.l mosquitoes were used for the bioassays. Eighty mosquitoes were exposed to each of the insecticides (5% malathion, 0.1% propoxur, 4% DDT, 0.75% permethrin and 0.05% Deltamethrin) with 20 mosquitoes used as control for each test. Mortalities in the control group were between 0-10% and these control mortalities were corrected for using Abbott's formula (Abbott, 1925). The summary of the susceptibility tests results are given in Table 4. The resistant ratio was obtained by comparing knockdown time in the wild mosquito population with those of the susceptible

strain. The data on mortality and knockdown time for the respective tests is presented in Appendix 11.

4.2.1 Malathion (5%)

Ninety eight percent (100%) mortality was observed from the susceptibility tests results, showing that the wild mosquito population is susceptible to malathion (5%). The knockdown times (KDTso) of 22.26 minutes and KDT95 of 33.52 minutes were recorded during the test. The susceptible strain test results indicated 100% mortality with KDT_{50} and KDT95 of 9.59 minutes and 28.83 minutes respectively.

4.2.2 Propoxur (0.01%)

Mortalities due to propoxur also showed the mosquitoes were susceptible to this insecticide. Ninety eight percent (98%) mortality was recorded for the wild population. A KDT50 of 31.10 minutes and KDT95 of 42.07 minutes were recorded for this population. The susceptible strain showed no resistance as expected, 100% mortality was recorded. The KDT50 and KDT95 were 9.59 minutes and 22.7 minutes respectively.

4.2.3 DDT (4%)

The susceptibility test results for this insecticide showed that the mosquitoes were highly resistant to this insecticide. The mortality test results for DDT (4%) was 13.75%. One hundred percent (100%) mortality was recorded for the susceptible 'Kisumu' strain. The KDT50 for the wild population was 134.07 minutes while the KDT95 was 221.07 minutes.

The susceptible strain as expected also showed susceptibility with knockdown times KDT_{50} of 24.23 minutes and KDT_{95} of 34.12 minutes.

4.2.4 Permethrin (0.75%)

The bioassay results for this insecticide showed that the population has developed resistance to permethrin. The corrected mortality for the tested population was recorded at 58.33%. The KDT_{50} and KDT95 for the wild population were 97.31 minutes and 154.37 minutes respectively. The 'Kisumu' strain on the other hand was susceptible to the insecticide. Hundred percent (100%) mortality was recorded with KDT50 and KDT95 of 9.59 minutes and 28.83 minutes respectively.

4.2.5 Deltamethrin (0.05%)

Susceptibility status of *An. gambiae s. I* to deltamethrin suggests that they are resistant to this insecticide with observed corrected mortality of 76.4%. The susceptible 'Kisumu' strain recorded 100% mortality to deltamethrin. A KDT50 of 55.47 minutes and KDT95 of 90.16 minutes were obtained from the time-mortality regression using probit analysis for the wild population. The susceptible strain recorded 8.93 minutes and 26.7 minutes for the KDTsoand KDT95 respectively.

Table 4. Resistance classification of *An. gambiae* s.l based on knockdown and mortality to various insecticides (WHO, **1998).**

Insecticide	**Corrected mortality**	**KDT** 50 **(minutes)**	95 % **C.I**	**KDT$_{95}$ (minutes)**	95 % **C.I**	**Resistance Status**
Malathion (5%)	98%	22.36	18.46-27.07	33.52	28.47 - 48.32	**S**
Propoxur (0.01%)	98%	33.10	32.38 - 34.35	42.08	40.15-45.29	**S**
DDT (4%)	13.75%	133.57	107.39 - 222.33	195.16	147.30- 359.23	**HR**
Permethrin (0.75%)	58.33%	97.31	87.33 - 113.32	154.37	133.03- 189.03	**R**
Deltamethrin (0.05%)	76.47%	55.47	53.09 - 58.58	90.16	84.44 - 97.46	**R**

C.I = Confidence interval KDT50 = Time taken for 50% of the test mosquitoes to be knocked down

KDT= Knockdown Time **KDT95=** Time taken for 95% of the test mosquitoes to be Knocked down

S= Susceptible **HR=** Highly resistant **R=**Resistant

4.3. Morphological Identification

Morphological identification using keys from Gillies and de Mellion (1968) and Service (1980) was conducted on all the mosquitoes used in the susceptibility tests. All the *Anopheles* mosquitoes were identified as *Anopheles gambiae* s.l.

4.4. Molecular Studies

The methods used in the molecular studies are Polymerase chain reaction (PCR) based. *An. gambiae* s.s species identification was done and positive results were digested using RFLP- PCR to determine M and S forms. Detection of the presence of the *kdr* mutation in the wild population was also conducted.

4.4.1 PCR Identification of *Anopheles gambiae* s.s

Sixty *An. gambiae* s.l mosquitoes that were identified morphologically were used for the species identification. All the individuals were identified as *An. gambiae* s.s with band size 390 bp. An example of gel electrophoregram showing the diagnostic PCR product band sizes is shown in figure 16.

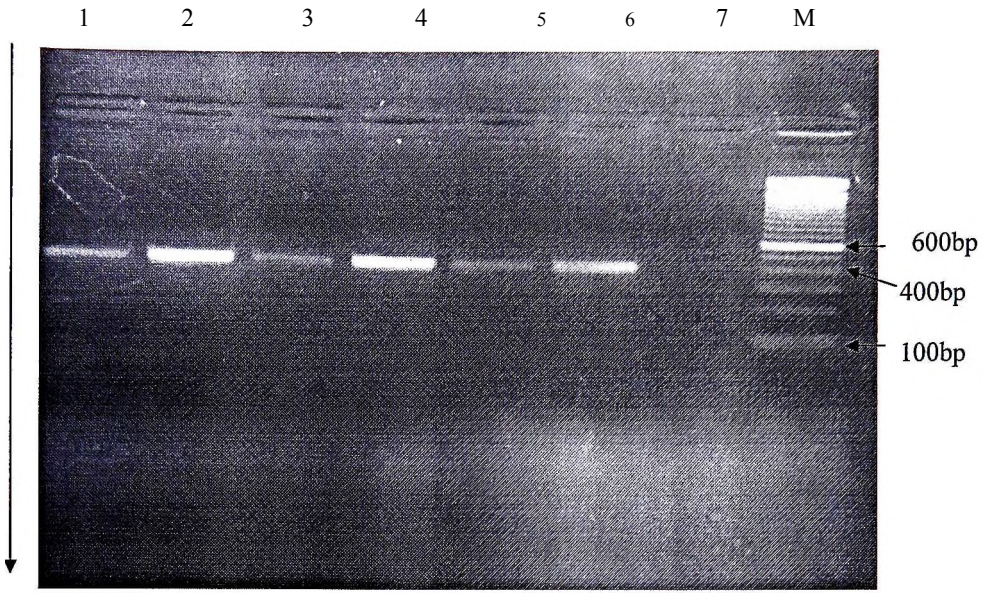

Figure 16. A gel electrophoregram of PCR products *of Anopheles gambiae* s.s.

PCR products electrophoresed in a 2% agarose gel stained with ethidium bromide.

Lane 1-6 = *Anopheles gambiae* s.s, Lane 7 = negative control,

Lane M= lOObp ladder.

► Direction of flow

4.4.2 Identification of the Molecular Forms of *Anopheles gambiae* s.s

Of the 60 mosquitoes identified as *An. gambiae* s.s, 30 were used for M and S form identification. All the 30 identified were M form with band size 367 bp. An example of the gel electrophoregram which was electrophoresed in a 2% agarose gel stained with ethidium bromide is shown in figure 17.

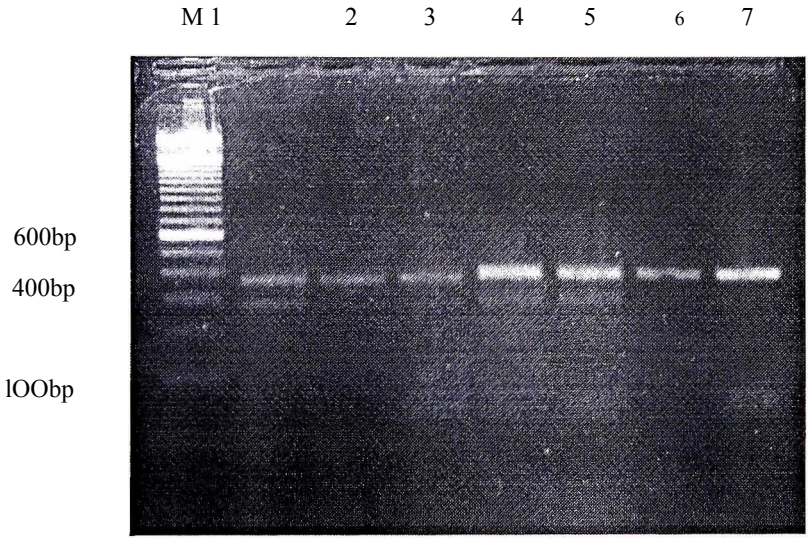

Figure 17. Identification of molecular forms of *Anopheles gambiae* s.s

. Lane M= 100 bp ladder, Lane 1-7= *Anopheles gambiae* s.s. M form.

_____ ---► Direction of flow

4.4.3 Allelic Frequency Distribution of *kdr* Mutation

Detection of the presence of *kdr* alleles in the population was carried out on 20 *Anopheles gambiae* s.s. All members of the tested population were found to be *kdr'* with band sizes 195 bp and 293 bp indicating presence of the *kdr* mutation that confers knockdown resistance. An example of the gel electrophoregram which was electrphoresed in a 2% agarose gel stained with ethidium bromide is shown in figure 18.

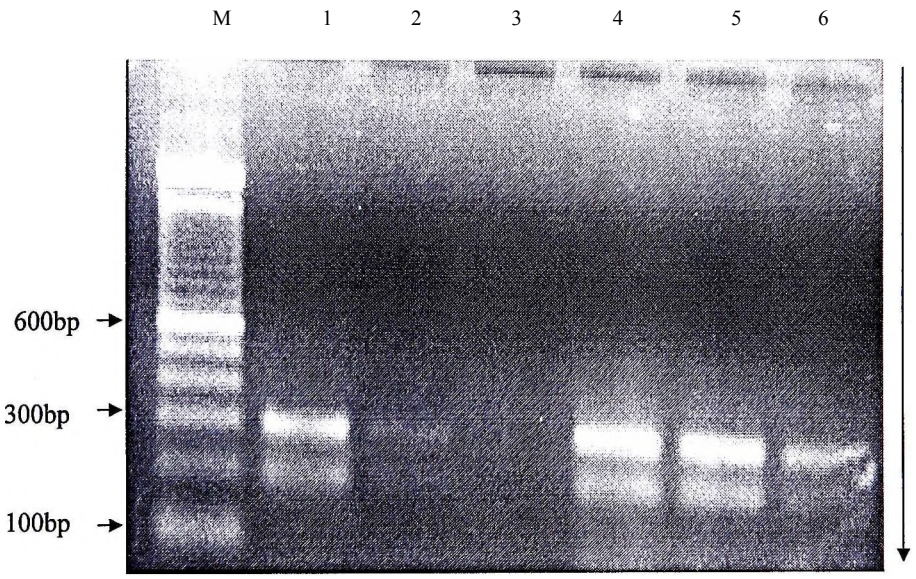

Figure 18. Gel electrophoregram for detection of *kdr* gene in PCR products from *Anopheles gambiae* s.s.

Lane M= 100 bp ladder, Lanes 1, 2,4, 5, 6= kdr+, Lane 3= Negative control

----------► Direction of flow

4.5.1 Insecticide Residues in Water Samples

Insecticide residues were detected in all the water samples collected from the fields. Lamdacyhalothrin, permethrin and cypermethrin were detected by **GC-MS** in varying quantities (appendix IV). Residue levels quantified for lamdacyhalothrin in the three plots were 1.5 ng/ml in plot A, 16.5 |ig/ml in plot B while no residue was detected from plot **C.** The residue levels for permethrin were 18.5 p.g/ml in plot **A,** 4.5 |ig/ml in plot B and 5.5 Hg/ml in plot **C.** For cypermethrin levels of 3.0 ng/ml was recorded for plot A, 1.0 ng/ml in plot B and 3.0 ng/ml in plot **C** (Figure 19).

4.5.2 Insecticide Residues in Soil Samples

Insecticide residues were also detected in all the soil samples collected from the fields (appendix IV). Lamdacyhalothrin, permethrin and cypermethrin were found in varying quantities. Residue levels quantified for lamdacyhalothrin in the three plots were 12 |ig/mg in plot A, 3.5 fig/mg in plot B and 1.5 ng/mg in plot **C.** The residue levels for permethrin were 1.5 (ig/mg in plot A, 4.5 ug/'mg in plot **C** while no residues were detected in samples obtained from plot B. Levels of cypermethrin in soil were 3.0 ng/mg in plot A, 1.0 |ig/mg in plot B and 3.0 (ig/mg in plot **C** (Figure 20).

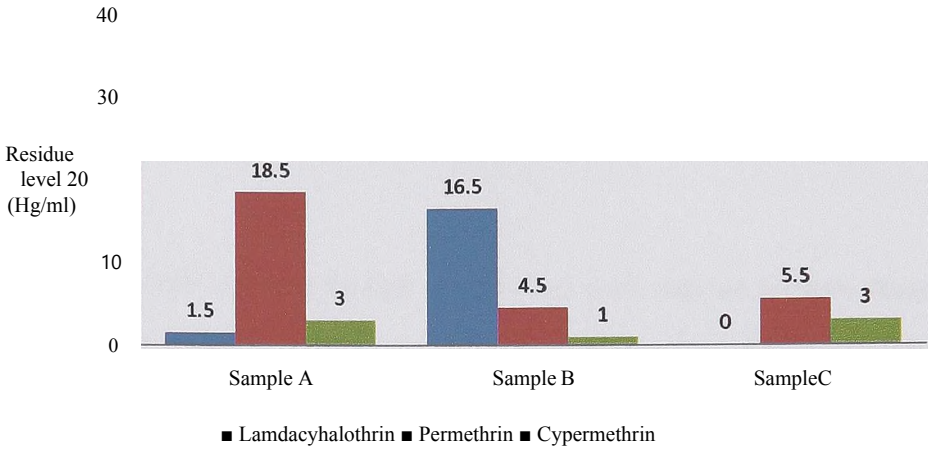

Figure 19. Insecticide residue levels in water samples from rice farms in Okyereko.

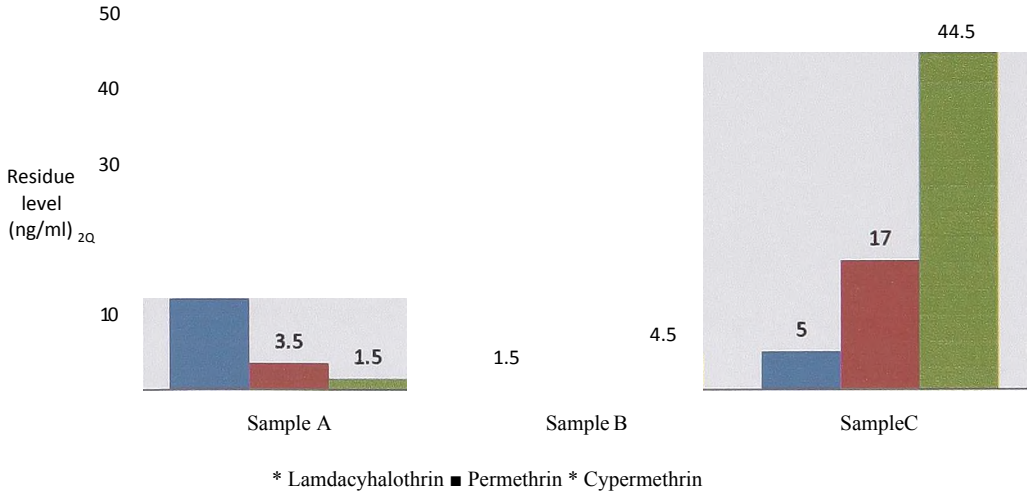

Figure 20.. Insecticide residue levels in soil samples from rice farms in Okyereko.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Insecticides are very crucial in the fight against insect pests; they are relatively cheap, readily available, require less labour and can give results relatively quickly. However the use of chemical control requires adequate knowledge, skills and facilities which are very necessary to achieve the required results. Farmers in developing countries such as Ghana lack some of the basic principles that encompass insecticide usage. Hence there is a host of misuse of insecticide during the various processes involved in acquisition and usage of chemicals. This misuse of insecticides can lead to environmental pollution and resistance in non-target organisms such as vectors of diseases that are exposed to the chemicals and accumulation as residues in organic products such as fruits and milk.

The role of agricultural insecticides in the development of resistance to vectors of diseases such as the *Anopheles gambiae* has been a subject of debate. According to various studies resistance development to vectors such as mosquitoes has been found to be rapid in areas of high insecticide use in crops (Adasi *et al.,* 2000; Ben-Mahmoud, 2008; Diabate *et al.,* 2002). This is because the insecticides used in crop protection are the same type of chemicals used in public health. This study is aimed at investigating the link between insecticide use, residue accumulation in mosquito breeding grounds and the development of resistance to *Anopheles gambiae,* the main malaria vector in Africa.

In okyereko, rice farmers are confronted with problems associated with stemborers and caseworms. The main strategy employed to control these pests according to the survey results is the use of pyrethroids insecticides such as permethrin and lamdacyhalothrin. The use of only pyrethroid insecticides in the area may indicate a shift in farmers' choice. According to studies by Ntow (2006) farmers in Ghana use all the main classes (organophosphate, carbamate, organochlorines and pyrethroids) of insecticide in vegetable and rice production. The year round cultivation of rice due to the presence of an irrigation system means that pyrethroid insecticides are also continuously applied to control the caseworms and stemborers. This suggests that there is a tendency for rapid buildup of insecticide residues in soil and water within and around rice farms and probably also in rice products.

The level of education among rice farmers in the area is low as indicated by the survey (38% of the respondents did not go to school). This conforms to studies done by Gerken *et al.* (2001) in Ghana in which only an average of 11.8% of the farmers in the survey areas had education higher than senior secondary. This lack of adequate knowledge and skills is a constraint to farmers when it comes to decision-making on rate and time of insecticide application in the field. Thus there is a high tendency of misuse of insecticide among farmers especially during handling storage and application. Majority (78%) of farmers interviewed were above 40 years. This suggests that farming is not attractive to young people in the area and this is likely to affect the future development of agricultural productivity especially rice production.

Farmers do not effectively follow safety measures during storage, transportation and application of insecticides. The survey showed that many farmers do not put on complete protective clothing during field application of insecticide. Similar findings were obtained by Aboagye (2002) among pineapple farmers in Southern Ghana and Odhiambo (2005) among vegetable farmers in Greater Accra. They usually use normal farm clothing during application and this can have devastating effect on their health. It is therefore not surprising that some farmers are exposed to the danger of acute poisoning and general ill health (Gerken *et al.,* 2001). The reasons forwarded for not putting on protective clothing during application of insecticides include unavailability of the materials, unaffordable price, and clothing considered uncomfortable in hot weather. The lack of adequate training on the use and care of the knapsack insecticide spray which is the equipment used in field spraying also compounds the problem.

Resistance to insecticides by mosquito vectors of malaria is a major challenge to the fight against the disease. This is because vector control which is a major WHO strategy in fighting the disease is insecticide based. The fact that the group of insecticides (pyrethroids) recommended by WHO for ITN and indoor residual spraying is also the same type used by farmers in controlling rice pests in the area should be a source of concern. This is because the irrigation system in the area provides an excellent breeding environment for mosquitoes such as the *Anopheles gambiae* the main malaria vector in West Africa. These vectors breeding in the rice farms are exposed to insecticide treatment

each time the chemical is used against the target crop pests. Continuous exposure of early stages of the vector to insecticide is likely to create rapid resistance development among the mosquito population in the area.

The WHO susceptibility tests for determining resistance in mosquitoes employ discriminating dosages that are set at double the insecticide dose that give 100% mortality of the least susceptible *Anopheles* mosquitoes (Penilla *et al.,* 1998). Based on WHO criteria, susceptibility is defined as mortality rates greater than 98%, marginal susceptibility as mortality rates between 80-97% and resistance as mortality rates below 80%, 24 hours after exposure to the insecticide. The bioassays conducted on *Anopheles* mosquitoes collected from rice farms in Okyereko indicate development of resistance to permethrin (58.33% mortality) and deltamethrin (76.47 % mortality). This is probably due to the fact that this same group of insecticide is continuously used by farmers to control crop pests in the area. Similar results have been reported among *Anopheles gambiae* populations in cabbage farms in Accra (Adasi *et al.,* 2000, Adeniran, 2002, Achondu, 2005 and Ben- Mahmoud, 2008,) and in shallot farms in Anloga in the Volta Region (Obeng, 2007). According to Yadouleton *et al.* (2010) *Anopheles gambiae* population from Southern Benin were found to be highly resistant to permethrin and DDT especially in areas of high insecticide use by vegetable farmers in urban areas for example.

Though DDT was not reported to be currently used by farmers in the area against crop pest, the susceptibility results indicate strong resistance to DDT with only 13.75% mortality recorded. This may have occurred due to the similarity in the mode of action of DDT and Pyrethroids, which is basically on the voltage dependent sodium ion channels (Brooke *et al.,* 1999 and Bloomquist, 1996). Resistance to DDT may have been as a result of previous use of this insecticide by farmers in the area before it was banned. Farmers may also possibly be still using the insecticide in the area even though it is on the list of banned insecticides in Ghana. The high susceptibility of the *Anopheles* mosquitoes in the area to malathion (organophosphate) (98% mortality) and propoxur (carbamate) (98% mortality) is indicative of the fact that farmers have not been reported to be using these groups of insecticides as was seen in the survey results.

The high knockdown times for permethrin, deltamethrin and DDT is probably indicative of the presence of knockdown resistance among the *Anopheles gambiae* population in the area. Knockdown resistance *(kdr)* has been associated with pyrethroids and DDT. It occurs due to the presence of the *kdr* mutation gene which confers resistance to these groups of insecticides. It has been widely reported in *Anopheles gambiae* found breeding in many agricultural areas especially in cotton farms where pyrethroids and DDT are being used against crop pests (Mouchet, 1998). Ben Mahmoud (2008) and Achondu (2005) reported high knockdown times for *Anopheles gambiae* popultions found breeding in cabbage farms within the Accra metropolis.

The results obtained from the molecular studies were all positive for *Anopheles gambiae* s.s indicating that it is the dominant species in the area. This confirms results from studies done by Okoye *et al.* (2005) in (Okyereko). The *Anopheles gambiae* s.s is the most effective vector of malaria and its dominance prevalence in the Okyereko rice farms is a potential threat to the health of the people and communities within Okyereko and the surrounding villages.

The *Anopheles gambiae* s.s M form was also found to be predominant in the area according to the PCR-RFLP results. The M form of the *Anopheles gambiae* s.s is normally located in water logged areas and in lowland breeding sites. For example in The Gambia the M-form was present in all sampling areas during both sampling seasons and was the most frequent taxon found during the rainy season in the western and in the central parts of The Gambia, alluvial flooded areas where rice is continuously cultivated (Caputo *et al.,* 2008). Robert *et al.* (1985) by a study of the distribution of *An. gambiae* s.s. cytotypes in the rice field area of Vallee du Kou in Burkina Faso in 1984 showed a predominance of the M chromosomal form. With the progress in molecular genetics, this distribution has been recently updated by Diabate *et al.* (2000) still pointing to the predominance of the M molecular form corresponding to the M chromosomal form. The results on the molecular form identification test showed similar trend to that of Okoye *et al.* (2005) in the area in which out of 150 *Anopheles gambiae* s.s more than 90% were identified to be M forms.

The entire twenty *Anopheles gambiae* s.s M form tested carried the *kdr* resistant gene. This result was surprising since the resistant gene is normally found to be more prevalent in the S form relative to the M form. This may however suggest that there is possibly a high prevalence of *kdr* gene among the *Anopheles gambiae* s.s population breeding in the area. This, nonetheless, is consistent with studies by Achonduh (2005) in cabbage farms in the Accra metropolis and Diabate *et al.,* (2002) in cotton and rice farms in Burkina Faso, where the *kdr* gene was found to be highly present in both M and S forms of *Anopheles gambiae* s.s in the respective areas. It is possible that the continuous use of pyrethroids against crop pests is a likely factor causing high prevalence of *kdr* resistance gene among the mosquito population.

Insecticide (pyrethroids) residues were detected in both water and soil samples. In some instances residues levels in soil was higher than those in water but the contrary was observed for some insecticides (Figure 4.15 and Figure 4.16). This was surprising since the residues level in soil is normally expected to be higher in soil the than in water FAO (2000). The results obtained from the residue analysis may be due to the fact that insecticides, especially pyrethroids, being unstable, degrade at different rates depending on the microclimate they are in. In addition, the area is flooded with water daily due to the presence of an irrigation system and many factors such as environmental and edaphic can contribute differently to the fate of these residues. Furthermore, the fate of pesticides in soil and water environments is influenced by the physico-chemical properties of the pesticide, the properties of the soil and water systems (presence of clay materials, organic matter, pH), the climate, biology, and other factors (Singh *et al.,* 1999).

El Beit *et al.* (1981) have reported that the persistence of a pesticide in the soil depends on the soil's pH, texture, structure and microorganisms presents.

The levels of insecticide residues detected in both water and soil samples are indicative of the fact that the sites were the samples were collected from are contaminated with insecticides. The mosquitoes breeding in these contaminated areas are therefore continuously exposed to doses of the insecticide that could exert selection pressure which can lead to the development of resistance over time. Vectors breeding in contaminated farms are exposed to lethal and sub-lethal doses of insecticides whenever crops are treated (SIMA, 2003).

The mortality and knockdown ratios obtained from the susceptibility tests, the high proportion of *kdr* allelic mutation in the tested *Anopheles gambiae* s.s individuals and the residue levels of pyrethroids detected in water and soil samples is suggestive of the fact that the use of pyrethroids in the area may be contributing to the development of resistance among the mosquito population in Okyereko.

5.2 Conclusions

The study reveals that farmers in Okyereko do not have adequate knowledge, skills and equipments needed for proper and optimal insecticides usage. The dominant species of Anopheles in area was found to be the *Anopheles gambiae* s.s and its M form is also the

main form found breeding in Okyereko rice fields. The *Anopheles gambiae* population in the area is susceptible to malathion and propoxur but showed resistance to pyrethroids (permethrin and deltamethrin) and DDT. There was a high presence of the *kdr* mutation gene among the *Anopheles gambiae* s.s population breeding in the area. Insecticides (pyrethroids) residues were detected in both soil and water samples collected from the rice fields.

5.3 Recommendation

- The study should be conducted in other irrigation systems in comparison with non irrigation areas.
- The number of individual mosquitoes to be tested for presence of *kdr* mutation gene should be large enough to give a clearer picture.
- Public health workers should work with agricultural extension officers to help sensitize farmers to the importance of proper insecticide usage in crops.

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APPENDICES

APPENDIX 1

Preparation of **standard solutions for molecular studies**

Standard solutions were prepared using double distilled water. Solutions were autoclaved where appropriate at 12 b/sq in for 15 minutes in Eyela Autoclave (Rikikkaki, Tokyo).

Solutions used in DNA Extraction

Bender buffer	0.1 M NaCl, 0.2 sucrose, 0.1M Tris- HCL pH 7.5, 0.05M 0.05 M EDTA pH 9.1, 0.5% SDS. Stored at 4 °c.
KAc (8M)	60 ml of KAc and 11.5 ml glacial acetic acid in 28.5 ml distilled water.
RNase	10 mg/ml. Sterilized by filtration and stored at 20 °C
TE (pH 8.0)	10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0). Stored at room temperature.
TE + RNase (5 ^g/ml)	5 jal of Rnase (10 mg/ml) solution, 995 p.1 of TE (pH 8.0). Stored at room temperature.

2. Solutions for gel electrophoresis

I. Agarose gel

10X TAE buffer	242 g Tris base, 75.1 ml glacial acetic acid, 100 ml of 0.5 m EDTA. pH adjusted to 7.7 (with glacial acetic acid) and the volume made to 1000 ml with dd H_2O.
EtBr (10 mg/ml)	1 g of EtBr was completely dissolved in 100 ml dd H_2O and stored in the dark at room temperature.

II. Gel loading buffer

Bromophenol blue	20% (w/v) Ficol, 25 m M EDTA, 2.5% (w/v) Bromophenol blue. Stored at 4 °C

APPENDIX 11

WHO SUSCEPTIBILITY TEST RESULTS

Malathion (5%) Susceptibility test

Date of testing: 20/01/10_____ Relative humidity: 71-80% Temperature26-30°C

Exposure time	Number knockdown per replicate			
	1	2	3	4
	0	2	0	0
10	0	4	0	0
15	0	6	1	1
20	4	14	1	2
30	15	18	13	13
40	19	20	19	19
50	20	20	20	20
60	20	20	20	20
80				
Total exposed	20	20	20	20
Total in control	20	20	20	20
Mortality (after 24hrs holding period)	20	20	20	20
Observed mortality (%)	100	100	100	10(
Control mortality (%)	5	5	5	5
Corrected mortality *(%)*	98	98	98	98

Average Mortality: 100%

Propoxur (0.1%) Susceptibility tests

Date of testing: 3/12/09 _____ Relative humidity: 71-80% Temperature26-30°C

Exposure time	Number knockdown per replicate			
	1	2	3	4
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	0	0	1	0
30	3	5	4	5
40	14	15	15	14
50	17	18	18	17
60	18	20	18	18
80				
Total exposed	20	20	20	20
Total in control	20	20	20	20
Mortality (after 24hrs holding period)	20	20	20	20
Observed mortality (%)	100	100	100	10(
Control mortality (%)	5	5	5	5
Corrected mortality (%)	98	98	98	98

Average Mortality: 100%

Permethrin (0.75%) Susceptibility tests

Date of testing: 18/02/10_____ Relative humidity: 71-80% Temperature 26-30°C

Exposure time	Number knockdown perreplicate			
	1	2	3	4
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	0	0	0	0
30	0	1	0	0
40	1	2	1	1
50	3	3	2	1
60	3	4	3	2
80	4	4	4	3
Total exposed	20	20	20	20
Total in control	20	20	20	20
Mortality (after 24hrs holding period)	14	13	11	12
Observed mortality (%)	70	65	55	60
Control mortality (%)	10	10	10	10
Corrected mortality *(%)*	66.7	61.1	50	55.

Average Mortality: 58.35%

Deltamethrin (0.75%) Susceptibility tests

Date of testing: 11 /04/10 Relative humidity: 71-80% Temperature 26-30°C

Exposure time				
	1	2	3	4
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	1	1	1	1
30	1	1	2	4
40	5	4	3	6
50	7	5	5	8
60	12	9	7	13
80	15	11	12	13
Total exposed	20	20	20	20
Total in control	20	20	20	20
Mortality (after 24hrs holding period)	16	16	14	18
Observed mortality (%)	80	80	70	90
Control mortality (%)	15	15	15	15
Corrected mortality *(%)*	76.5	76.5	64.7	88.2

Average Mortality: 76.5%

DDT (4%) Susceptibility tests

Date of testing: 10/12/09_____ Relative humidity: 71-80% Temperature26-30°C

Exposure time	Number knockdown perreplicate			
	1	2	3	4
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	0	0	0	0
30	0	0	0	0
40	0	0	0	0
50	0	0	1	1
60	0	0	1	1
80	0	1	2	1
Total exposed	20	20	20	20
Total in control	20	20	20	20
Mortality (after 24hrs holding period)	3	3	3	2
Observed mortality (%)	15	15	15	10
Control mortality (%)	5	5	5	5
Corrected mortality *(%)*	15	15	15	10

Average Mortality: 13.75%

Percentage average knockdown time and 24 hr mortality for different insecticides (WHO, 1998)

Insecticide class	Chemical	% Concen -tration	No of mosquito -es per cup	No of replicat- es	Average % knockdown at 15min	Average % knockdown at 30min	Average % knockdown at 60min	Average % knockdown at 80min	Average % 24hr mortality	[Control mortality	Corrected mortality (Abbot)©
Organophosphate	Malathion	5%	20	5	8%	59%	100%	-	100%	2	98%
Carbamate	Propoxur	0.1%	20	5	0%	17%	92.5%	-	100%	2	98%
Organochlorine	DDT	4%	20	5	0%	0%	2.5%	5%	13.75%	1	13.75%
Pyrethroids	Permethrin	0.75%	20	5	0%	1%	15%	52.5%	62.5%	2	58.33%
Pyrethroids	Deltamethrin	0.05%	20	5	0%	10%	51.25%	65%	80%	3	76.47%

© = Abbot formulae

APPENDIX 111

SURVEY OUESTIONAIRE

ASSESSMENT OF INSECTICIDE USE PATTERNS AMONG RICE FARMERS IN OKYEREKO, CENTRAL REGION.

Personal Information

1. Operational area/village ....
2. Gender Male / female
3. Age a) < 20 yrs (b)20 - 30 yrs (c) 31-40 yrs (d) 41-50 yrs (e) >50 yrs
4. What is your educational background?

   a) Primary (b) Junior secondary (c) Senior secondary (d) Tertiary (e) None

Land tenure / Rice production

5. How would you classify your production level?

   a) Small scale (b) medium scale (c) large scale

6. Land ownership

   a) own (b) leased (c) family (d) shared

7. How many rice farms do you cultivate per season?

   a) 1 (b) 2 (c) 3 (d) 4 (e) >4

8. Complete the table below.

Farm	farm area (ha)	No. of years under rice cultivation

9. Do you rotate rice with other crops on the farms?

Incidence of pests/Choice and source of insecticides

10. Do you encounter pest(s) problems? Yes/no Please list the pests encountered

11. Which pest(s) poses the biggest problem?.....

12. Do you use insecticides to control the pest(s) yes/ no

13. Which insecticide(s) do you normally use on your rice farm(s)?

14. Why do you use this/these insecticide(s)?

15. When do you buy your insecticide(s)?

16. Where do you obtain your insecticide(s)?

17. Where do you store your insecticide(s) before and after use?

(a) in my house (b) in a farm house (c) Others specify.....

Insecticide Application/ Safety

18. Do you apply insecticide at the nursery stage? Yes/ no. If yes, how do you apply the insecticide? a) by hand (include broom, brush, home made pump, cup etc)

(b) Knapsack (c) motorized sprayer

19. How do you apply insecticides in the field?

a) by hand (include broom, brush, home made pump, cup etc) (b) knapsack sprayer (c) motorized sprayer (d)tractor sprayer (e) others specify.....

20. Who does the application? (more than one answer is possible)

a) myself (b) spouse (c) children (d) hired labourers (e) extension agents

(g) others specify.....

21. What type of clothing do you put on when applying insecticides?

22. Indicate the protective clothing you use during insecticide application.

Protective material	Used during application
Long trouser	
Overall	
Gloves	
Respirator	
Wellington boot	
Goggles	
Hat	
Long sleeve (shirt)	

Others specify,

23. How do you formulate your insecticides?

a) by following directives on the insecticide container label

b) following routine practices

c) getting assistance from other farmers

d) assistance from extension agents

e) others

specify.....

24. What is the rate of concentration used for the different insecticides? Please refer to table

Insecticide	Concentration rate

25. Do you use a mixture of insecticides to control pests? Yes / no. if yes what insecticides do you mix and in what proportion. Please refer to table

Name of insecticide	Proportion in the mixture

26. How do you dispose off empty insecticide containers?

a) re-use them (b) destroy them (c) others specify.....

Knowledge of insecticide use

27. Where do you obtain information on insecticide use?

a) label on the container (b) extension agents (c) commercial dealers (d) Radio/television (e) others specify.....

28. Did you receive any special technical training on insecticide application? Yes/ No

If yes give details.

29. How often do you get technical advice on the use of insecticide?

30. From which organization do you get technical advice? (More than one answers possible) a) Extension agents (b) commercial retailers (c) NGOs (d) other farmers

(d) Others specify.....

31. Which recommendation(s) do you find useful? (More than one answers possible)

a) frequency of application (b) dosage (c) type of insecticide (d) safety

(e) others specify.....

32. Have you encountered any problem(s) with insecticide use? Yes/ no. If yes, what are the problem(s)? a) fell sick (b) phyto-toxicity (c) poisoning (d) pollution of water bodies (e) others specify..... ....

33. How do you know this problem was as a result of insecticide use?

34. How do you know what dosage to apply?

35. Do you observe a pre-harvest interval after applying insecticides? Yes / no. if yes how long is this period, a) 1 week (b) 2 weeks (c) 1 month (d) > 1 month

36. When do you decide to apply insecticides?

a) after appearance of pests (b) calendar spray (c) After advice from expert

(d)Others specify.....

37. Do you keep farm records on your insecticide use pattern? Yes/no

Give reason(s) for your

answer.....

Mosquito and malaria problems

38. Do you encounter mosquito problems at home? Yes /no. if yes how do you control them?

39. How effective is the control measure(s)?

a) very effective (b) moderately effective (c) less effective (d) no effect

40. How often do your family members have malaria?

41. What is your preferred malaria treatment?

a) doctor's prescription (b) Pharmacy (c)herbal prescription

(d) others specify.....

42. How much do you spent per treatment?

Please specify.....

43. Do you frequently change brands of insecticide (aerosol) you use to spray mosquitoes?

Yes/no. Give reason(s) for you answer

44. Do you think mosquitoes are resistance to the insecticides used to control them? Yes/ no. Give reason(s) to your answer.

45. Do you have a water body within or around your farm? Yes / no. If yes do you observe mosquitoes breeding there? Yes / no

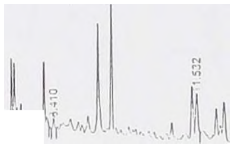
APPENDIX IV

Residues Analysis Results

Weights of Residues after Solid Phase Extraction

Sample	Weight of vial with residue (g)	Weight of empty vial (g)	Weight of residue (mg)
Ai Soil	6.2054	6.1999	5.5
A2 Soil	6.0855	6.0826	2.9
Bi Soil	5.9887	5.9875	1.2
B_2 Soil	6.0208	6.0171	3.7
Ci Soil	6.1263	6.1209	5.4
C_2 Soil	6.0108	6.0069	3.9
A1 Water	5.9645	5.9606	3.9
A_2 Water	5.9618	5.9600	1.8
Bi Water	5.7723	5.7705	1.8
B_2 Water	5.9665	5.9647	1.8
Ci Water	6.0398	6.0383	1.5
C_2 Water	5.8360	5.8342	1.8

Sample A2: water sample from Okyereko rice Helds, sif



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Manual Sample

. Dace: 6/24/10 S:2S ?M Calculacicn Dace: 6/29/10 3:35 AM

: Paul
on: GS3
C : C? 3800
: Middle = ECD

Dececror Type 3800 (10. VolC3)
Bus Address **44**
Sample Race **10.00 Hz**
Run Time 20.980 min

Icscacicn Version 5.41 ** 02460-3090-cS5-01f4 **

1.03 cn/min Accenuacion = 142 Zero Offsec 79%
0 . 000 End Time 20.980 min Min / Tick 1.00

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In j eccion Dace: 6/24/10 4-. 12 ?M Calculacicn Dace: 6/29/10 3:25

Operacor : Paul
Workscacion: GS3
Inscr.menc : C? 3 8 00
Channel : Middle = ECD

Dececcor Type 3 3 00 (10 Voles)
Bus Addres 3
Sample Race 10.30 Hz
Run Time 20.980 mm

*» GC Workscacion Version 6.41 *» 02460-3090-c55-01f4 * *

Chare Speed = 1.03 cm/min Accsr.uacion = 15 3 Cffsec =
SCarc Time = 0.000 min End Time = 20.980 min Min

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\ :zj Manual Sample

in Dace: 6/20/10 1:24 PM Calculation Dace: 6/29/10 3:36 AM

: Paul Detector Type 3800 (10 Voles)
ion: GS3 3us Address 44
DC : CP 3S00 Sample Race 10.00 Hz
: Middle = ECD Run Time 20.980 min

rkstation Version 6.41 * 02460-3090-C65-01

1.03 cm/min Attenuation = 162 Zero Offset = 78%
0.000 min End Time = 20.980 min Min / Tick = 1.00

Sample C2 : water sample from Okyereko rice Helds, site 3 .

Sample ID Manual Sample

Operator	Paul	Detector Type	3800 (10 Volts)
Workstation	GSB	Eus Address	
Instrument	CP 3800	Sample Rate	10.00 Hz
Channel	Middle = ECD	Run Time	20.980 min

GC Workstation Version 6.41 ** 02460-3090-c65-01f4 **

Chart Speed = 1.03 cm/min Attenuation Zero Offset = 32%
Start Time = 0.000 min End Time 20.980 mi; Min / Tick = 1.00

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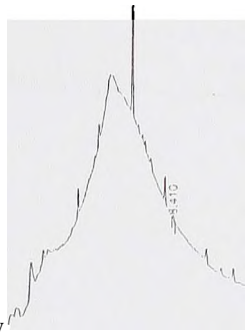

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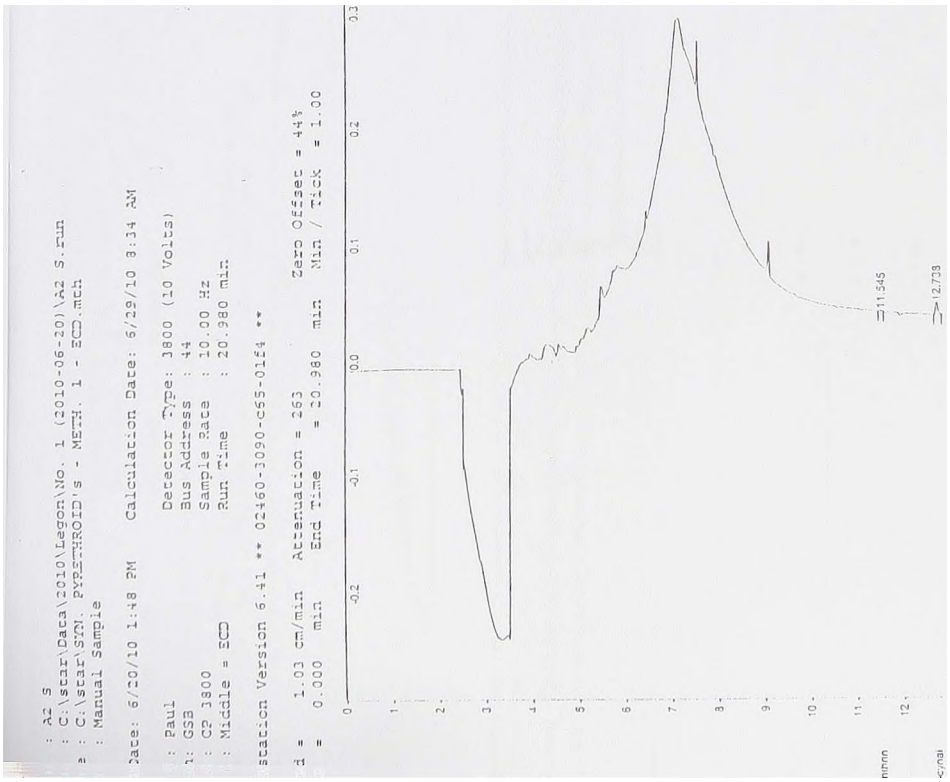
T! Sample A2: Soil sample from Okyereko rice iidcls, sile



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Ma; jal Sample

: (/27/10 1:2 Calculation Dace: 6/29/10 8:35 AM

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Detector Type	3800 (10 Volts)
Bus Address	44
Sample Rate	10.00 Hz
Run Time	20.980 min

Lor Version 6.41 02460-3090-c65-01f4 «*

1.03 cm/min Attenuation = 233 Zero Offset= : 55%
c .000 min End Time = 20.980 min Min / Tick = 1.00

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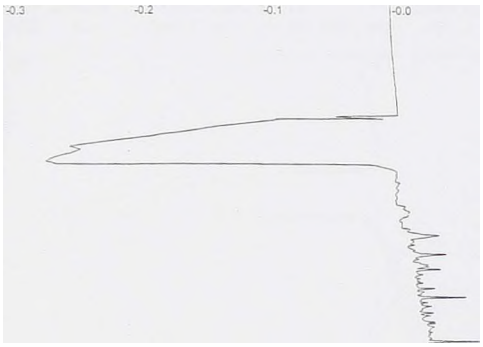

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mple B2: Soil sample from Okyeicko ricc fields, site 2 -

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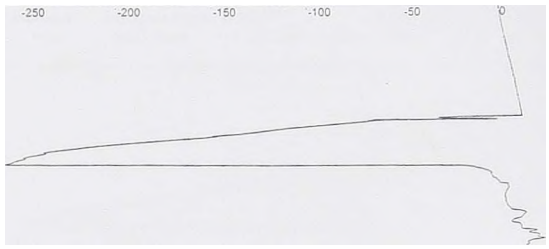
:: \car\SYU. PYRETHROID■ 3 -
lan aal Sample

6 /24/10 5:49 PM Calculation Date: 6/29/10 8:37 AM

IUI	Detector Type	3800 (10 Volts)
IB	Bus Address	
> 3	Sample Rate	10.00 Hz
.dd Le = ECD	Run Time	20.980 min

.or Version 6. 02460-3090-c65-01f4 **

1.03 cm/min Attenuation 158 Zero Offset
O.000 min End Time = 20.980 min Min / Tick



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Manual Sample

n Dace: 6/24/10 5:02 PM Calculacion Dace: 6/29/10 8:36 AM

: Paul Dececcor Type 3800 (10 VolCs)
ion: GS3 Bus Address 44
nc : CP 3800 Sample Race 10.00 Hz
: Middle = ECD Run Time 20.98 0 min

rkscacion Version 6.41 02460-3090-c65-01f4 **

eed = 1.03 cm/min Accenuacion = 256 Zero Offsec = 49%
me = 0.000 min End Time = 20.980 min Min / Tick = 1.00

Sample C2: Soil sample from Okyereko i ice lields, site 3

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  Title
  Run File        C:\star\Data\2010\Legon\No. 1 (2010-06-20)\C1 S.run
  Method File     C:\star\SYN- PYRETHROID's - METH. 1 - ECD.mch
  Sample ID       Manual Sample

j Injection Dace: 6/24/10 4:38 PM        Calculation Dace: 6/29/10 3:36 AM

| Operator : Paul                          Decector Type    3800 (10 Voles)
[ Workstation: GSB                         Bus Address      44
  Instrument : CP 3800                     Sample Race      10.00  Hz
  Channel : Middle = ECD                   Run Time         20.980 min

     GC Workstation Version 6.41         02460-3090-G 5 5-01f 4

  Chare  Speed       1.03     cm/min Attenuation = 219                 Zero Offset
  Scart Time        0.000 min            End  Time     = 20.980 min Min / Tick
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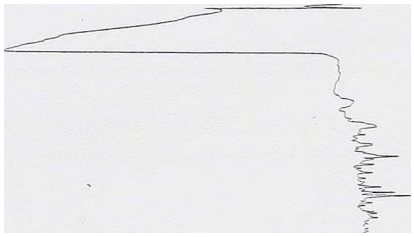

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