DISSIPATION STUDIES OF RIDOMIL GOLD (METALAXYL M) AND ACTARA 25WG (THIAMETHOXAM) PESTICIDES ON IRISH POTATOES (*SOLANUM TUBEROSUM*) AND SNOW PEAS (*PISUM SATIVUM*) IN NYANDARUA COUNTY.

BY

BONFACE NDERI KAMAU

[I56/76027/2014]

A Thesis Submitted for Examination in Partial Fulfillment of the Requirements for Award of the Degree of Master of Science in Chemistry of the University of Nairobi.

2017

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

Signature……………………………..…….. Date……………………………………..

BONFACE NDERI KAMAU

[I56/76027/2014]

This thesis has been submitted for examination with our approval as University supervisors.

Signature……………………………..…….. Date……………………………………..

Prof. Shem O. Wandiga

Department of Chemistry, University of Nairobi.

Signature……………………………..…….. Date……………………………………..

Dr. Vincent O. Madadi

Department of Chemistry, University of Nairobi

DEDICATION

This thesis is dedicated to my Mother and my family: my dear wife Njeri and my daughters Wanjira and Muthoni for their splendid support, inspirational love and encouragement.

ACKNOWLEDGEMENT

My heartfelt joy goes to the Almighty God who granted me the grace and strength to complete this thesis.

I thank my supervisors, Prof. Shem Wandiga and Dr. Vincent. O Madadi for their valuable and generous contribution towards the completion of this work. I also appreciate the members of the pesticide research group: Enock, Dickens, Susan, Dorice, Masese, Vane, Emily and all 2014 M.Sc Chemistry students; Kinyua, Dickens, Martin and Isemeki for their moral support.

I thank the Pest Control Products Board (PCPB) for providing me with the standard Metalaxyl-M and Mr. Koigi from Kephis for assisting me with the standard Thiamethoxam.

I thank all members of academic and technical support staff in the Department of Chemistry for their input in developing my project proposal and throughout my research. I also sincerely thank members of my family for their patience and moral support throughout my years in school.

I am very grateful to the Almighty God for granting me life, good health, grace and strength to carry out my research work and prepare this thesis. To all of you, thank you and God be with you and bless you in all your endeavors.

ABSTRACT

The main objective of this study was to assess the dissipation rates of methyl *N*-(methoxyacetyl)-*N*-(2, 6-xylyl)-D-alaninate (ridomil gold; metalaxyl M) and 3-(2-chloro-1, 3-thiazol-5-ylmethyl)-5-methyl-[1, 3, 5]-oxadiazinan-4-yllidine (nitro) amine (Actara 25WG; thiamethoxam) pesticides applied on potatoes and snow peas in Nyandarua County where potatoes and snow peas farming are ventures that are helping the small scale farmers to boost their income. However, the yields are profoundly affected by fungi and insect pest. The average yield of <10 t/ha is low compared to 40-50 t/ha obtained in countries with well-developed potato production system in America and Europe. Pesticides are very important in agriculture in Kenya because of decreasing quality and quantity of crop production due to diseases and pest infestations. Whereas pesticides are capable of controlling crops and livestock diseases and pests, they are also poisons that can affect non-target organisms such as human and wildlife. Farmers in Nyandarua County had registered complaints about lack of effectiveness of the Ridomil Gold used to control fungi on potatoes. In addition, stringent regulations in the European market limit the maximum residual levels on exported products that have seen the rejection of Kenyan snow peas from the EU market due to high residues of pesticides.

Samples were collected and Soxhlet extracted using organic solvents, and quantified for Metalaxyl M using GC-MS while Thiamethoxam was analysed using GC-FID. The half-lives of Metalaxyl M and Thiamethoxam dissipation were calculated using the Langmuir-Hinshelwood kinetic model equation and regression curves drawn to get the rate constants. Results obtained showed Metalaxyl M dissipation half-life of 3.13 days and 3.61 days in potatoes and soil, respectively, while Thiamethoxam had a dissipation half- life of 9.34 days and 13.13 days in snow peas and soil, respectively.

The concentration of on thiamethoxam on snow peas on the pre-harvest interval (PHI) day was lower than both the EU and Codex MRLs. Metalaxyl M residues on potato leaves after day 7 were below the limit of detection, clearly indicating that after day 7 the pesticide is not effective to control the early and late blight. All the potato samples collected from the three markets (Njabini, Kiburu and Tulaga) open air markets had Metalaxyl M residues below detection limit. The concentrations of Thiamethoxam residues during wet season were 0.44 mg/Kg, 0.41 mg/Kg and <LOD for Njabini, Kiburu and Tulaga, respectively, while during the dry season the levels were 0.49 mg/Kg, 0.46 mg/Kg and 0.43 mg/Kg, respectively. The results revealed higher pesticide concentrations during the dry season. The most commonly used pesticides in the region were organophosphates, carbamates and pyrethroids. Despite the fact that they are highly toxic compounds, most farmers in the study area did not adhere to the guidelines on the safe use of pesticides. The findings of this study revealed that lack of appropriate data on dissipation rates and proper use of pesticides contributed to ineffective control of pest and high maximum residual levels in post-harvest products.

TABLE OF CONTENTS

DECLARATION .... **II**

DEDICATION .... **III**

ACKNOWLEDGEMENT .... **IV**

ABSTRACT .... **V**

LIST OF TABLES .... **XII**

LIST OF FIGURES .... **XIII**

LIST OF ABBREVIATIONS .... **XV**

UNITS OF MEASUREMENT .... **XVII**

CHAPTER ONE **1**

1. INTRODUCTION **1**

1.1 GENERAL BACKGROUND OF THE STUDY 1

1.1.1 PESTICIDE REGULATION 4

1.2 STATEMENT OF THE PROBLEM 5

1.3 HYPOTHESIS 7

1.4 RESEARCH QUESTIONS 7

1.5 OBJECTIVES 7

1.5.1 Main Objective *7*

1.5.2 Specific Objectives *7*

1.6 JUSTIFICATION 8

CHAPTER TWO **10**

2. LITERATURE REVIEW **10**

2.1 CLASSIFICATION OF PESTICIDES 10

2.1.1 Carbamates *10*

2.1.2 Organophosphate Pesticides (OPs) 11

2.1.3 Organochlorine Pesticides (OCPs) 12

2.1.4 Organosulfurs 13

2.1.5 Synthetic Pyrethroids 13

2.2 METALAXYL M PESTICIDE 14

2.3 PHYSICAL AND CHEMICAL PROPERTIES OF METALAXYL M. 15

2.3.1 Structure of Metalaxyl M 15

2.3.2 Solubility of Metalaxyl M. 16

2.3.3 Environmental Fate in Soil 16

2.3.4 Stability of Residues in Stored Analytical Samples 16

2.4 PHYSICAL AND CHEMICAL PROPERTIES OF THIAMETHOXAM 16

2.4.1 Structure of Thiamethoxam 16

2.5 MANAGEMENT OF SYSTEMIC FUNGICIDES. 17

2.6 PESTICIDE TOXICITY 18

2.7 PESTICIDE USE IN KENYA 19

2.8 VEGETABLES GROWN AND THEIR YIELDS IN KENYA 21

2.9 PESTICIDE RESIDUES IN FOOD 23

2.10 PREHARVEST INTERVALS 28

2.11 SNOW PEAS GROWING 28

2.12 IRISH POTATO GROWING 29

CHAPTER THREE **31**

3. MATERIALS AND METHODS **31**

3.1 THE DESIGN OF THE STUDY 31

3.1.1 Field Survey 31

3.2 Dissipation Studies and Experiments 32
3.3 Residue Analysis 33
3.4 Preparation of Drying Agents, Column Materials and Solvents 34
3.5 Sample Processing 34
3.6 Sample Extracts Clean-up 35
3.7 Sulphur Removal 36
3.8 Recovery Tests 36
3.9 GC-MS Analysis and Quantification of the Extract Samples 37
3.10 Quality Assurance and Quality Control 37
3.11 Moisture Content Determination 38
3.12 Statistical Data Analysis 38
3.13 Physicochemical Parameters of Soil 38

CHAPTER FOUR 40

4. RESULTS AND DISCUSSION 40

4.1 Pesticides Use Survey in Kinangop Sub-County 40
4.1.1 Background information on Farming in the Three Sampling Areas. 40
  4.1.2 General information from Questionnaires Returned. 41
  4.1.3 Level of Education of the Respondents 42
  4.1.4 Age of the Respondents 43
  4.1.5 Pesticides Used in Kinangop Sub-County and their Classifications 43
  4.1.6 Factors Affecting Choice of Pesticide 44
4.1.7 Impacts of Pesticides on the Environment 45
  4.1.8 Training on pesticide management & safety 46
  4.1.9 Effectiveness of Pesticides 47

4.1.10 Training sessions on use of pesticides for farmers 48

4.1.11 Common Challenges of the Farmers 49

4.2 PHYSICOCHEMICAL CHARACTERISTICS OF SOILS FROM THE STUDY AREA 49

4.3 METHOD ACCURACY AND RECOVERY TESTS 50

4.4 QUANTIFICATION TESTS 51

4.5 Quantification of Thiamethoxam 53

4.6 DISSIPATION OF METALAXYL M IN POTATOES LEAVES 54

4.7 DISSIPATION OF METALAXYL M IN SOIL 58

4.8 DISSIPATION OF THIAMETHOXAM IN SNOW PEAS LEAVES 60

4.9 DISSIPATION OF THIAMETHOXAM IN SOIL 63

4.10 ASSESSMENT OF METALAXYL M RESIDUES IN POTATOES 65

4.11 ASSESSMENT OF THIAMETHOXAM RESIDUES IN SNOW PEAS 65

4.12 DISCUSSION 67

CHAPTER FIVE 70

5. CONCLUSIONS AND RECOMMENDATIONS 70

5.1 CONCLUSION 70

5.2 RECOMMENDATIONS 71

REFERENCES 73

APPENDICES 85

APPENDIX I 85

TABLE A1 TEST SOIL FERTILITY EVALUATION PARAMETERS AND PHYSICOCHEMICAL PROPERTIES 85

APPENDIX II 86

QUESTIONAIRE ON THE USE OF PESTICIDES IN KINANGOP, ... 86

APPENDIX III 92

TABLE A2: QUESTIONNAIRES ANALYSIS. 92

APPENDIX IV 95

TABLE A3: PHYSICAL AND CHEMICAL PROPERTIES OF METALAXYL M 95

APPENDIX V 97

TABLE A4: PHYSICAL AND CHEMICAL PROPERTIES OF THIAMETHOXAM 97

APPENDIX VI 98

TABLE A5: STRUCTURES OF DIFFERENT TYPES OF PESTICIDES 98

APPENDIX VII 102

TABLE A6: MAJOR SNOW PEAS AND POTATO PEST AND DISEASES. 102

APPENDIX VIII 105

PUBLISHED PAPER 105

LIST OF TABLES

Table 2. 1 Volume of pesticides imported from 2006/2007-2009/2010 into Kenya. 20

Table 2. 2 Major vegetables grown in the subsector 22

Table 4.1 Pesticides Used in Kinangop Sub-County and their Classifications 44

Table 4.2 Method Recovery, data in % for Metalaxyl M and Thiamethoxam Residues 51

Table 4.3 Results of Metalaxyl M Dissipation in Potatoes leaves in mg/Kg (Dry weight) 54

Table 4.4 Results of Metalaxyl M Dissipation in Soil (mg/Kg, dw) 59

Table 4.5 Results of Thiamethoxam Dissipation in snow peas (mg/Kg, dw) 61

Table 4.6 Results of Thiamethoxam Dissipation in Soil (mg/Kg, dw) 63

Table 4.7 Assessment of Metalaxyl M Residues in Potatoes mg/Kg (dry weight) 65

Table 4.8 Assessment of Thiamethoxam Residues in Snow Peas mg/Kg (dry weight). 66

LIST OF FIGURES

Figure 2. 1 Structure of Metalaxyl M (Active ingredient in Ridomil Gold) 15

Figure 2. 2 Structure of Thiamethoxam (active ingredient in Actara 25WG)..........................17

Figure 3. 1 Map representing the study area (Kinangop, Nyandarua County). 32

Figure 4.1 Percentage of Questionnaires returned 41

Figure 4.2 Gender of the respondents..41

Figure 4. 3Level of education of the respondents 42

Figure 4. 4Age of Respondents 43

Figure 4.5 Factors affecting the choice of pesticide by farmers...45

Figure 4. 6Effects of Pesticides to the Environment 46

Figure 4. 7 Training on pesticide management & safety 47

Figure 4.8 Rating of pesticides used. 48

Figure 4.9 Training sessions on use of pesticides 49

Figure 4. 10 Calibration curve of Metalaxyl M standard 52

Figure 4.11 GC/MS ion chromatogram for Metalaxyl M standard..52

Figure 4. 12 Chemical structure and ionic mass Spectra for Metalaxyl M standard...............53

Figure 4. 13 Calibration curve of Thiamethoxam standard 53

Figure 4. 14Trends of Metalaxyl M Dissipation in Potatoes leaves...55

Figure 4. 15Regression curve for disappearance of Metalaxyl M from Potatoes 57

Figure 4.16 Chromatogram of Metalaxyl M Residues in treated Soil 58

Figure 4. 17Trends of Metalaxyl M Dissipation in Soil..59

Figure 4. 18 Regression curve for disappearance of Metalaxyl M from Soil...........................60

Figure 4.19 Trends of Thiamethoxam Dissipation in Snow Peas leaves..................................61

Figure 4. 20 Regression curve for disappearance of thiamethoxam from snow peas 62

Figure 4. 21 Trend of thiamethoxam dissipation in soil 64

Figure 4. 22 Regression curve for disappearance of thiamethoxam from Soil 64

Figure 4.23 Trends of thiamethoxam residues in snow peas at three markets 66

LIST OF ABBREVIATIONS

ADI	Acceptable Dairy Intake
AEZ	Agro-ecological zones
a.i	Active Ingredient
ARfD	Acute Reference Dose
EAC	East Africa Community
EFSA	European Food Safety Authority
EIQ	Environmental Impact Quotient
EPRIP	Environmental Potential Risk Indicator for Pesticides
EU	European Union
EYP	Environmental Yardstick
FAO	Food and Agriculture Organization
GC-MS	Gas chromatography- Mass Spectrometry
GC-ECD	Gas Chromatography with Electron Capture Detector
GC-FID	Gas Chromatography with flame ionization detector
GDP	Gross Domestic Product
GOK	Government of Kenya
HCDA	Horticulture Crops Development Authority

IPEST	Pesticide Environmental Impact Indicator
IPM	Integrated Pest Management
LOQ	Limit of Quantification
MOA	Ministry of Agriculture
MRLs	Maximum Residual Levels
NTS	National Traceability System
PCPB	Pest Control Products Board
p-EMA	Environmental Performance Indicator
PERI	Pesticides Environment Risk Indicator
PHI	Pre-harvest Interval
RAR	Renewal Assessment Report
SPS	Sanitary and Phytosanitary
SyPEP	Systems for Predicting the Environmental Impact of Pesticides
WHO	World Health Organization

UNITS OF MEASUREMENT

µl	microliter
^{0}c	Degree Celsius
g	gram
Kg	kilogram
L	Liter
ml	milliliter
Ppm	parts per million

CHAPTER ONE

1. INTRODUCTION

1.1 General Background of the Study

Agriculture contributes about 25% to Kenya's national GDP (HCDA and MOA, 2014). About 75% of the work force depends on this sector either directly as farmers or indirectly through handling the farm produce (MTTI, 2007). The major agricultural sectors are livestock husbandry and crop production (GoK, 2010). Among the crops, horticulture is the third foreign exchange earner and also currently provides livelihood to over 500,000 farmers of whom 80% engage in small and medium-scale farming activities (Kenya Economic Survey, 2012). The main horticulture crops grown in the country are: flowers, fruits and vegetables (EPZA, 2005). Agricultural activities mainly take place in the highlands which are high potential areas, coastal regions which are medium potential areas and in the savannah AEZs (NEAP, 1994). Many places experience bimodal rain pattern particularly the Lake Basin and the eastern plateau (KMD, 2009). Long rains and short rains are experienced from March to May and from October to December, respectively. The western Kenya (Kericho, Kisumu, Kakamega, Kisii, and Eldoret) highlands have one rainy season which lasts from March all the way to September (KMD, 2009). The hottest time period falls from early February to end of March and the coldest is July and August (KMD, 2009). The major markets for vegetables are France and UK which constitute more than 30% of the market by volume (EPZA, 2005). In the recent past, the sales of fresh vegetables from Kenya have been on the rise annually. The major vegetables include French beans, sugar snap and snow peas among others (EPZA, 2005).

It is estimated that about 0.5 million people in rural areas and over 2 million people in the urban are employed by the horticulture industry (GOK, 2005).

The horticulture industry is the major consumer of pesticides. However, the over reliance on the use of pesticides has many consequences to national economy, health and environment (Gilden *et al.,* 2010). A lot of concern has been raised by FAO on ill health caused by the use of pesticides in the world (FAO, 1998). In addition, the export market customers demand low pesticide residues in horticultural products or a reduction in pesticide use (GOK, 2005). Furthermore, consumers are also concerned about the presence of pesticide residues in food stuff since these compounds have potential to harm non-target organisms. Pesticides are known to interfere with several physiological and developmental processes in living organisms including the fetus (Gilden *et al.,* 2010). They are also known to initiate asthma and cancer (Gilden *et al.,* 2010).

It is known that in the conditions of the contemporary agricultural production over 30% of the produce does not find its way to the consumer, whereas without the application of the chemical agents the loss would be doubled (Bursic' *et al.,* 2009). Therefore, whereas pesticides are generally important in agriculture, they have harmful effects to human health and wildlife and their presence in food ought to be strictly controlled (Vidal *et al.,* 2002).

In 2006 the number of people poisoned annually was estimated to about 1 million. Out of these, about twenty thousand died (WHO, 2006) in cases attributable to toxicity of the pesticides. These pesticides were used by farmers without using recommended protective clothing and without adequate knowledge about handling of these pesticides. According to PCPB (2005), many people do not consider how these pesticides are applied before blaming health problems on pesticides. Pesticide abuse and misuse is common in Kenya (PCPB, 2004). Misuse is due to low level of education and lack of resources to buy appropriate protective clothing (PCPB, 2004). Some of the misuse cases reported in Kenya include: non-registered products, smuggled, while others are banned products which are sold in open air markets, counterfeit products and sale of expired products with modified expiry date (PCPB, 2004).

In 2014, sales of vegetables declined from 57,000 to 51,351 tonnes and the decline was attributed to tough phytosanitary and sanitary measures taken by EU (HCDA & MOA, 2014). The horticulture earnings fell from $393.6 to $383.6 million in the year 2014 (HCDA & MOA, 2014). This was attributed to a number of factors which included erratic weather and economic partnership agreements whose signing was delayed between the two blocks (EU and EAC) (HCDA & MOA, 2014).

Horticulture products in unshelled pods which include beans and peas from the country destined to EU markets are subjected to strict controls. This was necessitated by increase in cases of produce which were reportedly contaminated. These controls are phytosanitary and sanitary (Okello *at al*., 2014). From January to April 2014, the levels of pesticide chemicals in these two products (beans and peas) were above the limit set by the EU. Forty five consignments from Kenya were intercepted from January to April 2014. This was an increase from 41 consignments intercepted from January to April 2013. They contained produce that was contaminated. Analysts warned that this could lower access to this EU market (EFSA, 2015). All imported farm produce should be below the set EU limit of the pesticide residues (MRLs) in food (Hanafi *et al.,* 2010). There is a pilot project meant to trace the movement of fresh farm produce from the farm to the consumer (Okero and Swinton, 2010). This will take the stakeholders and the government of Kenya 18-months from March 2015 to complete the project with the aim of enhancing transparency in the sub-sector of fresh produce.

Kenyan bean and peas farmers are required to implement pesticide controls and to prove that their processes meet international pesticide standards and compliance (Okello *at al*., 2014). In 2013, Kenya authorities failed to demonstrate to the EU how they monitor the use of pesticides using an effective system (Okello *at al*., 2014). This led to the EU imposing a 10% inspection fee on all exported beans and peas products. The regulation resulted in decline in exports to the EU markets that affected many farmers and resulted into substantial losses (Mwangi, 2013).

Potatoes and snow peas farmers use different methods to protect their crops from pests and diseases. The major diseases include: late blight, early blight and powdery mildew, whereas the major insect pests include: leaf-miner flies, blister beetles and leaf beetles that feed on and destroy their crops. In addition, farmers also experience the burden of controlling weeds that compete for nutrients with their crops. Most of the farmers use chemical pesticides to manage pests and diseases vectors.

Pesticides used in Kenya are registered after meeting a set of regulatory standards that ascertain their safety and effectiveness against the target pests and diseases. In most of the cases, farmers who follow label instructions and guidelines provided for pesticides attest to a reduced risk of pest damage and increase in quantity of produce, contributing to the country’s food self-reliance.

1.1.1 Pesticide Regulation

The first pesticide legislation recorded was in 1921 which was under the Public Act, Cap 242. The act that prescribed different preparations for eradicating ticks was legislated in April 1937. Although these legislations have been amended, the original prescriptions are still in the national legislations (Wandiga *et al*., 2003).

An agricultural industry voluntary precautionary scheme was adopted when the colonial government declared emergency rules (during struggle for independent). This scheme was meant to protect the employees from poisoning by chemical substances used in agricultural activities and other incidental and related matters. This is the basis of 1954 Ordinance of poisonous substances which was derived from 1952 UK Act (Wandiga *et al.*, 2003). At the Westminster was passed an Act on pharmacy and poisons in 1st of May 1957. Measures to control poisons and veterinary drugs were included in this Act. It also included rules on labeling and selling of poisons which included pesticides. When Kenya attained independence, an Act was passed on 11th May1965 by parliament for preventing food adulteration. It also included prevention of adulterating chemical substances and drugs as well as incidental and other connected matters.

In Cap 254 of this Act, particular attention was given to pesticides and a definition was given to the term ‘chemical substances’ which referred to a mixture of substances or any substance presented or sold for use as disinfectant, germicide, insecticide, detergent, antiseptic, vermicide and rodenticides. Tolerance levels were set in ppm for foodstuffs and pesticides which was done for the first time in history (Wandiga *et al.*, 2003; KLR, 2010).

Parliament passed another Act Cap 318 on Agriculture in 1955 on pesticides distribution and use. Other legislations included Act Cap 345 on animal food stuffs and fertilizer, the 1979 Act Cap324 (Protection Act) and another one of 1983 which is about control of pests. The aim of the 1983 Act was to regulate manufacturing and distribution of pest control products. It also addressed issues to do with how to export, import these products and organic function on animal and plants. Another legislation was Agricultural produce (export) Act Cap 319 of 1923 that provided for inspection of farm agricultural produce and their grading for export and the regulation of their manufacture and preparation.

1.2 Statement of the problem

Whereas agriculture contributes over 25% GDP, and directly or indirectly accounts for 70% of the formal employment (GOK, 2005), the presence of pesticide residues on the farm produce above recommended maximum residue levels, environmental contamination, effectiveness against the target pests, and toxicity of pesticides to non-target organisms remain issues of major concern. Chemical pesticides are used by farmers for economic management of livestock and crops, (Kovach *et al.*, 1992). In Nyandarua County, potatoes and snow peas farming are ventures that are helping the small scale farmers to boost their income. However, the yields are profoundly affected by fungi and insect pests.

In the recent past, farmers in Nyandarua, have been complaining about lack of effectiveness of some pesticide formulations such as Ridomil Gold used to control fungi on potatoes (PCPB, 2005). In addition, the stringent regulation in the European market limit the maximum residue levels on exported agricultural products (Matthews, 2006). For instance, the snow peas from Kenya have been rejected from European market due to high residues of pesticides (Matthews, 2006).

The main pesticides used in Nyandarua are Ridomil Gold (metalaxyl M), Actara 25WG (thiamethoxam), Tactic, Dithane (mancozeb) and Karate (lambda-cyhalothrin). The continued occurrence of pesticide residues above MRLs brings into question the level of application of good agricultural practice (GAP) by farmers. Heavy pesticide use occurs due to numerous pest attacks on the horticultural crops. They are used in agriculture to protect crops from destructive pests both in the field and during storage. However, when used, pesticides contaminate the environment and accumulate in the food chain thereby posing hazard to human health, for instance Metalaxyl M may affect the unborn child and Thiamethoxam may affect liver, kidney and reproductive systems (Blasco *et al*., 2003; Leong *at al*., 2007; PAN, 2001). Thus the residues remaining after harvesting may reduce the market value of the yield of high value crops.

1.3 Hypothesis

Potatoes and snow peas produced in Nyandarua County are compliant with the EU guidelines with regard to Ridomil Gold and Actara 25WG pesticide residues.

1.4 Research Questions

The questions that were addressed in this study were the following:

1) Does lack of appropriate data on dissipation rates contribute to ineffective control of pest and diseases?

2) Does lack of proper use of pesticides contribute to high maximum residue levels in post-harvest products?

1.5 Objectives

1.5.1 Main Objective

The main objective of this study was to determine the dissipation rates of metalaxyl M and thiamethoxam pesticides on potatoes, snow peas and soil in Nyandarua County.

1.5.2 Specific Objectives

The specific objectives of this study were:

(i) To identify pesticides used in potatoes and snow peas farming in Nyandarua county and the level of awareness among the farmers.

(ii) To determine dissipation rates of Ridomil gold (methyl *N*-(methoxyacetyl)-*N*-(2, 6-xylyl)-D-alaninate) on potatoes.

(iii) To determine dissipation rates of Actara 25WG (3-(2-chloro-1, 3-thiazol-5-ylmethyl)-5-methyl-[1, 3, 5]-oxadiazinan-4-yllidine (nitro) amine) on snow peas.

(iv) To determine dissipation rates of metalaxyl M and thiamethoxam pesticides on farm soil in Nyandarua County.

(v) To assess the levels of selected pesticides in market products of potatoes and snow peas.

1.6 Justification

Use of pesticides to protect vegetation has become indispensable in Kenya. However, the effectiveness of the pesticides applied depends upon their physicochemical properties, environmental conditions and farmer adherence to the manufacturer specifications which have been tested and proven effective. The persistence and the level of environmental contamination of any chemical are measured by analyzing residues. Systematic research need to be done to help cope with the changing dynamics of international market by determining the levels of pesticide residues in horticulture products. Supervision trials are carried out by manufactures of different pesticides targeted for use on different vegetables and fruits in many countries. In Kenya, manufacturers either use imported active ingredients to locally formulate pesticides or import pesticides as finished final product. Data generated from the country of origin is only accepted by default. This is notwithstanding the fact that pesticides behave differently under different environmental conditions. There is rising concern on local farming practices of pesticide application and the harvesting of treated crops. This concern is on the possibility of there being high level of residues in farm produce.

Most of the research done on pesticides concentrate on the pollution of air, water and soil by pesticide residues, but studies on the amount of residual on crops produce are limited to crops which are produced on large scale.

Research on residual levels on potatoes and snow peas which are produced by small scale farmers will provide data on the potential risk to man and raise awareness for the regulator to look into mitigation measures. It will also help consumers to make informed decision when purchasing food for consumption. Further, dissipation studies of pesticides applied on potatoes and soil will provide data on the potentials losses of the pesticides and hence explain the cause of ineffectiveness to protect the crops against fungi and diseases.

CHAPTER TWO

2. LITERATURE REVIEW

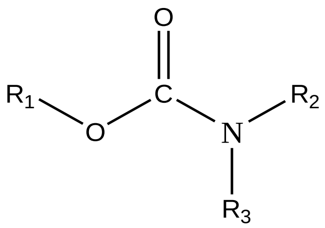
2.1 Classification of Pesticides

Pesticides are substances used to control pests as well as attract, destroy and prevent pests (Oudejans, 1991). There are two main classifications of pesticides, inorganic and organic pesticides (Oudejans, 1991). Inorganic pesticides are those with no carbon atoms and are obtained from material elements that occur naturally. They dissolve in water, are non-volatile and generally stable. Most inorganic pesticides contain arsenic, cyanide, mercury and thallium, but the presence of such metals make pesticides persistent and bioaccumulative (Hassall, 1990). On the other hand, organic pesticides are mainly synthetic compounds containing either aliphatic or aromatic hydrocarbon chains. They consist of organochlorines, organophosphates, orgonosulfates, carbamates and pyrethroids depending on the element(s) bonded to the hydrocarbon system (Wasswa, 2008).

2.1.1 Carbamates

Carbamic acid (NH_2COOH) is the compound from which carbamates are derived. Carbamic acid and carbamate ester (ethyl carbamate) are inter-related (structurally) functional groups and can be chemically inter-converted (Dorough and Casida, 1964).

Carbamates have groups attached to the central carbonyl carbon. R_2 is always an aromatic or aliphatic moiety.



The major difference among the carbamate pesticides is in the functional group attached at R_1. For instance, carbamate insecticides have R_1 as an ethyl group, herbicides have R_1 as an aromatic group, whereas fungicides have R_1 as a benzimidazole moiety (Dorough *et al.,* 1964). Some of the known carbamates are carbaryl, carbofurans and aldicarbs (Appendix VI Table A5). Biologically, carbamates resemble the organophosphates in their activity. They inhibit the cholinesterase enzyme required for nerve function in animals (USEPA, 2009). Some carbamates are also suspected carcinogens. Carbamates are hydrolyzed slowly in neutral and mildly acidic aqueous surroundings, but in the presence of alkali, they decompose rapidly (USEPA, 2009).

2.1.2 Organophosphate Pesticides (OPs)

These pesticides are phosphate esters which are derived from phosphoric acid. They comprise of a central phosphate atom and three organic side chains (R). Two of these organic side chains are usually ethyl or methyl, whereas one is more specific for a given pesticide (Briggs, 1992). Examples of OPs include; dichlorvos, dimethoate, and diazinon (Appendix VI Table A5). These pesticides are chemically unstable, less-persistent, but highly toxic to man and vertebrate animals. This group of pesticides has virtually replaced the persistent organochlorine compounds (Briggs, 1992).

Organophosphates are highly potent compounds used mainly as insecticides especially in food crops. They are very toxic and more often involved in acute poisoning than other classes of pesticides (Collins, 2006; Mansour, 2004). OPs exert their toxic activity by inhibiting the enzyme acetyl cholinesterase, which is the enzyme responsible for the hydrolysis of acetylcholine, a neurotransmitter that conducts nerve impulses across neuromuscular junction in the nervous system of vertebrates as well as insects. Inhibition of this enzyme causes accumulation of Acetylcholine (Ach) leading to generalized cholinergic action and muscles which eventually lead to paralysis, respiratory failure and death (Podolska and Napierska, 2006; Guilermino *et al.,* 2004). Chronic exposure of humans to low doses of pesticides through air, water and food may lead to chronic toxicity due to accumulation of residues in the body over a long period of time. Possible health problems associated with chronic pesticide toxicity include cancer, congenital malfunction, neurological disorders, infertility, impotence, immunological disorders, liver and kidney damage, skin alterations and worsening of existing health conditions (Jobling *et al.,* 1995). Acute and sub-acute toxicity may also arise from exposure to high doses among people who are directly involved in the manufacture, formulation, mixing and application of pesticide or in suicide and homicide cases (Jobling *et al.,* 1995). Human exposure may be through dermal contact, inhalation or accidental ingestion (Turgut, 2007). Symptoms of acute toxicity vary with individual chemical involved but may generally include dizziness, headache, sweating, fatigue, numbness, vomiting neurological effects, liver and kidney damage, coma or death (Turgut, 2007). Despite their benefits to man, pesticides are poisons and must be properly used to minimize human exposure and reduce health risks. Therefore, government regulation of pesticide use all over the world and analysis of pesticide residues in food is one way of monitoring effectiveness of the set regulatory systems.

2.1.3 Organochlorine Pesticides (OCPs)

Organochlorine pesticides are a large class of multipurpose chlorinated hydrocarbon chemicals (Briggs, 1992). They break down slowly in the environment and accumulate in the fatty tissues of animals. Thus, they stay in the environment and food web long after being applied (Idowu *et al.,* 2013). DDT, now banned globally because of its harm to the health of wildlife and people, is a notable example of an organochlorine pesticide. Many organochlorine pesticides are endocrine disrupting chemicals, meaning they have subtle toxic effects on the body’s hormonal systems (Lemaire *et al.,* 2004). Endocrine disrupting chemicals often mimic the body’s natural hormones, disrupting normal functions and contributing to adverse health effects. They are very toxic organic compounds, persist in the environment and have the potential for long range transport, posing a serious threat to the environment and its habitats at remote places (Vesna *et al.,* 2001). Organochlorine pesticides (OCPs) include dichlorodiphenyltrichloroethane (DDT), 1,1-dichloro-2,2-bischlorophenyl)(4-ethane) *p,p'*-DDD, methoxychlor and 2,2-bis *p*-chlorophenyl 1,1-dichloroethylene- *p,p'*-DDE (Appendix VI Table A5).

OCPs were used widely to protect crops, livestock, buildings and households against a variety of pests such as ticks, locust, termites and mosquitoes (Idowu *et al.,* 2013). Currently, most of these pesticides have been banned, except a few which are under restricted use (Idowu *et al.,* 2013). Following the ban, large stocks of obsolete OCPs are still in possession by individual farmers, households and government agencies. The probability of these chemicals being released into the environment is very high posing a high risk to animals and human health.

2.1.4 Organosulfurs

Organosulfurs have sulfur in their structure as the central atom (Briggs, 1992). Their mode of action is by disrupting the target organism's metabolism. They have low toxicity to insects and mammals and as a result used for selective purposes (Briggs, 1992). They are characterized by their toxicity to young and adult insects which is a valuable property. They also cause irritation to the eyes, ears and nose. The common examples are aramite, tetradifon, and tetrasulf (Appendix VI Table A5).

2.1.5 Synthetic Pyrethroids

Pyrethrin is a natural insecticide extracted from Chrysanthemum cineraria folium (pyrethrum)-the crude flower dust (Kegley and Hills, 2007). The synthetic pesticide pyrethroids are derivatives of pyrethrins which were designed to improve the biological activity of the active principal of the natural pesticide (Kegley and Hills, 2007). Pyrethroids synthesised before 1970 were very sensitive to sunlight, as their molecules split under UV light making them unsuitable for agricultural use but effective for indoor insect pest control (Chapman *et al.,* 1981). Since 1970s, synthetic pyrethroids with a better photo-stability and low volatility have been produced to suit both agricultural and indoor uses. This class of pesticides poisons the target by contact and causing paralysis (Chapman *et al.,* 1981). These compounds have low mammalian toxicity, but are highly toxic to insects and aquatic organisms. The common pyrethroids are deltamethrin and tetramethrin (Appendix VI Table A5).

2.2 Metalaxyl M Pesticide

Metalaxyl M (Mefenoxam) is one of the racemic mixture of Metalaxyl. This is the R-enantiomer which is the active form of Metalaxyl (EFSA, 2015). Metalaxyl M is more reactive than Metalaxyl, and is more effective at half the rate of Metalaxyl. The single isomer (Metalaxyl M) was developed by Novartis (Syngenta). It is authorized in Europe under regulation (1107/2009) of the EU agrochemical regulation. It is sold combined with Mancozeb or copper which are protectant fungicides. It is one of the systemic fungicides. Metalaxyl M is used to treat seeds, foliar and soil. One of its major uses is treatment of phytophthora (potato blight) and in vines it is used to treat penospora. Other crops where Metalaxyl M is used include tobacco, fruits (e.g. citrus), vegetables and peas.

In 2015, the EFSA (European Food Safety Authority) was given a mandate to update the assessment of Metalaxyl in accordance to Regulation (EC) No396/2005 of article 43, by the European Commission. They were to take into account the two enantiomer of Metalaxyl. The study derived the following conclusions.

The Metalaxyl M and Metalaxyl toxicological profile Directive 91/414/EEC, resulted in ADI (Acceptable Daily Intake) of 0.08 mg/Kgbw per d and acute reference dose (ARfD) of 0.5 mg/Kg bw. Four groups of crops (leafy, cereals, tuber and roots, and fruit crops) and three different types of applications, soil, foliar, and seed treatment were evaluated on all uses of Metalaxyl M and Metalaxyl. 0.01 mg/Kg was used as the (LOQ) limit of quantification on validated analytical methods for all plants. All MRL proposals were derived from the use of Metalaxyl M. The report also concluded that it could not be excluded that the use of Metalaxyl could lead to elevated residue levels compared to those obtained for Metalaxyl M (EFSA, 2015).

2.3 Physical and chemical properties of Metalaxyl M.

2.3.1 Structure of Metalaxyl M

The figure below shows the structure of Metalaxyl M.

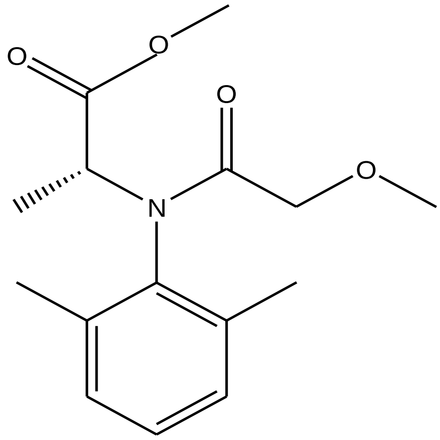


Figure 2. 1 Structure of Metalaxyl M (Active ingredient in Ridomil Gold)

The IUPAC name for Metalaxyl M is methyl *N*-(methoxyacetyl)-*N*-(2, 6-xylyl)-D-alaninate. The CAS methyl *N*-(2, 6-dimethylphenyl)-*N*-(methoxyacetyl)-D-alaninate. Chemical formula: $C_{15}H_{21}NO_4$, whereas the molecular weight is 279 g/mol (FAO, 1982).

2.3.2 Solubility of Metalaxyl M.

Metalaxyl M is completely miscible in toluene at 25^0C. It is also completely soluble in dichloromethane, ethanol, ethyl acetate, acetone, n-octanol, whereas the solubility in hexane at 25^0C is 59 g/l. (FAO, 1982). Other physical and chemical properties of Metalaxyl M are presented in Appendix IV Table A3.

2.3.3 Environmental Fate in Soil

Environmental fate of Metalaxyl is affected by factors such as temperature, its biological activity and the concentration of residues of Metalaxyl M (EFSA, 2011). The half-lives of disappearance of Metalaxyl M from the soil ranges from five to thirty five days (EFSA, 2011). The residues are obtained mostly in the top 10 cm of soil. However, most studies suggest that when Metalaxyl M is used during sowing or to treat seeds, very little or no residue remain in the soil at the time of harvest (EFSA, 2011).

2.3.4 Stability of Residues in Stored Analytical Samples

When the substrate is stored in the freezer, there is no evidence of epimerization. This is because Metalaxyl M in the substrate is very stable for 2 years (period of storage) (EFSA, 2011).

2.4 Physical and chemical properties of Thiamethoxam

2.4.1 Structure of Thiamethoxam

The Figure 2.2 below shows the structure of Thiamethoxam.

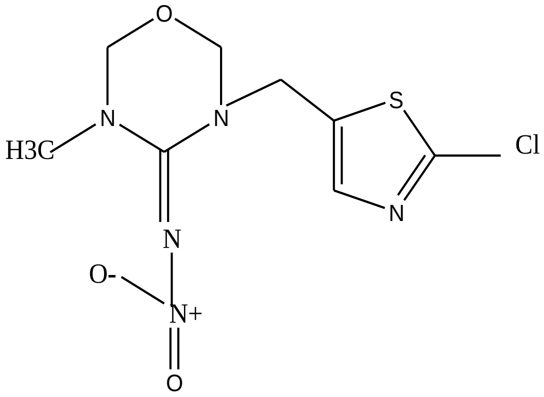


Figure 2. 2 Structure of Thiamethoxam (active ingredient in Actara 25WG)

The IUPAC name of thiamethoxam is 3-(2-chloro-1, 3-thiazol-5-ylmethyl)-5-methyl-[1, 3, 5]-oxadiazinan-4-yllidine (nitro) amine. The chemical formula is $C_8H_{10}ClN_5O_3S$ whereas the molecular weight is 291.71 g/mol.

Thiamethoxam is stable in organic solvents which are used in biocidal products. In addition, the breakdown products have also been found to be stable in the environment (MacBean, 2010). Other physical and chemical properties of thiamethoxam are presented in Appendix V Table A4.

2.5 Management of Systemic Fungicides.

Metalaxyl M is an example of systemic fungicides and when indiscriminately used may lead to development of resistance from resistant of fungus. For instance *Peronospoles infectans* is a resistant strain of Metalaxyl that was reported in Bangladesh (Dey and Ali, 1994).

Metalaxyl, ofurance, oxadxyl and benalaxyl are systemic phenyl amide fungicides that control diseases that are caused by peronospoles, exclusively. They show excellent antifungal activities which include, eradicative, protective and curative (Schwinn and Staub, 1987).

Between 1981 and 1984 there was an increase in resistant isolates of Metalaxyl called phytophthora. During this period there was suspension of phenyl amide fungicides in Ireland

and the Netherlands. A study carried out in Estonia for 3 consecutive years 2003, 2004 and 2005 showed that the resistant isolates of Metalaxyl detected increased in frequency. The isolates were more frequent in 2005 than in 2004 and 2003. It was found that there was fluctuation of the proportions of resistant isolate of Metalaxyl from year to year, a factor which was attributed to the fact that the quality of resistant isolates of Metalaxyl had increased as compared to previous reported results (Runno and Koppel, 2005). Based on the findings by Runno and Koppel, (2005) it was concluded that resistant isolates of Metalaxyl were unlikely to completely displace the isolates that are sensitive. This was only if Metalaxyl is used following the recommendation.

They noted that if at the beginning of the fungicide treatment Metalaxyl is applied at maximum two times, it can be used to effectively control late blight in potatoes. This means that Metalaxyl can still be used in spite of occurrence of these Metalaxyl-strains. To restrict resistant strains of Metalaxyl from developing, application rules must be followed to avoid reducing the fungicide’s efficacy (Runno and Koppel, 2005).

2.6 Pesticide Toxicity

Pesticides are plant protection products that increase the yield of agricultural produce. They also help in storage of food crops by extending the storage life. They improve the quality of food crops by destroying the disease vectors and pests (Fernandez-Alba and Garcia-Reyes, 2008). These are mainly fungi, insects, rodents and weeds. Whereas pesticides are effective at eliminating these pests (Matthews, 2006), the application of pesticides in the environment leads to dispersal into air, soil and water where they also cause toxicity to non-target organisms such as human, soil organisms, wildlife and crop plants in the immediate area. A certain proportion of pesticides also mixes with air and surface water (Gilden *at al.*, 2010) where it can be taken up by human beings and wildlife. It can also be deposited on plants or on the soil and further leak into ground water. Aquatic organisms may also consume the pesticides in the surface water and through sedimentation of the pesticides in surface water may go into organisms remaining in sediments. According to WHO (2008), there are several physical and chemical properties of the pesticides that determine their persistence. These properties include the pesticide's degradation rates, its rate of deposition, its partition coefficients and the environmental characteristics. For the production of enough vegetables, fruits and grains for the country, pesticides are indispensable (Atsukoa and Toshio, 2006). Judicious and appropriate use of pesticides is essential for the production of crops and management of diseases and pests in an environmentally sustainable manner (Jeyanthi and Kombairaju, 2005). In this regard, evaluation of safe use of pesticides must consider not only the parent compounds, but also the impact of the metabolites formed, persistence as well as their toxicological properties. Contamination of the environment as a result of farmers not using pesticide properly as stipulated by the manufacturer has been widely documented (Lartiges and Garrigues, 1995).

2.7 Pesticide Use in Kenya

The economy of Kenya largely depend on agriculture. This has raised the demand for pesticides for economic management of crops and livestock against a myriad of pests and disease vectors. In the late 1990's there was a sharp increase in demand for pesticides due to introduction of large-scale farming of horticultural crops (Harris, 2000).

There has been growing concerns in the recent years about the improper use of agrochemicals which can create environmental and human hazards (Harris, 2000). The need to produce farm produce which are not damaged by pest and which do not contain any pesticide residue is a major challenge facing the horticulture industry in Kenya. Concerns due to risks of non-target impacts of pesticides is increasing globally as shown by increasingly more stringent standards on pesticide residue levels (Howard *et al.*, 1991; Mullen, 1995; Mathews, 2006). Pesticides cause atmospheric contamination, affect human health by causing acute and chronic illnesses and also contaminate water both surface and ground (Howard *et al.,* 1991; Mullen, 1995; Mathews, 2006). Pesticides are associated with reproductive disruption, disruption of endocrine system and delays in neurodevelopment. Other hazards include; distress of the respiratory system, cancer and also lowering on immunity (Gilden *at al.*, 2010).

There are many factors that are used to determine how pesticides impact the environment. Environmental conditions are some of the factors; and these include type of soil, site characteristics and geological formation. Other factors that determine the environmental impact include; pesticide dosage, method and frequency of application and the physical-chemical properties of pesticide active ingredient (Reus *et al.,* 1999).

Table 2. 1 Volume of pesticides imported from 2006/2007-2009/2010 into Kenya.

Category	2006/2007		2007/2008		2008/2009		2009/2010	
	Amount of pesticide in tons	Value of pesticide in million (Kshs)	Amount of pesticide in tons	Value of pesticide in million (Kshs)	Amount of pesticide in tons	Value of pesticide in million (Kshs)	Amount of pesticide in tons	Value of pesticide in million (Kshs)
Insecticides	2475	1181	2887	3909	2995	2079	3181	2493
Fungicides	3190	1251	2651	602	2340	3153	2415	3874
Herbicides	1859	324	2289	206	2933	944	1840	939
*Others	1225	362	1330	191	1413	1167	1396	918
TOTAL	8749	3443	9157	4908	9681	7343	8832	8232

**Source: PCPB Annual Report, 2009/2010.

*Including growth regulators, surfactants and bio pesticides

From the above table, approximately 8,832 metric tons of pesticides valued at Kshs 8.2 billion were imported into the country in 2009/2010. During this period, insecticides represented the largest quantities of pest control products imported, indicating an increase in importation cost from the previous years. In addition to synthetic pesticides, dried flowers of pyrethrum are used to produce natural pesticide called pyrethrin. Kenya is one of the major producers of this natural pesticide. However, 95% of the crude pyrethrum is exported to more environmentally conscious developed countries, where it earns a premium price, leaving Kenya to import the cheaper toxic synthetic pesticides (PCPB, 2005).

In developing countries, there are few studies carried out on negative impacts of using pesticides (Van der werf and Zimmer, 1998). There are several environmental impact assessment models which have been used to assess the impact of pesticides which include:

i) Environment Impact Quotient (EIQ).

ii) Pesticide Environmental impact Indicator (Ipest) (Van der werf and Zimmer, 1998).

iii) Environmental Yardstick (EYP)

iv) Environmental Performance Indicator of pesticides (p-EMA) (Lewis *et al.,* 2003).

v) Environmental Potential Risk Indicator for Pesticides (EPRIP).

vi) System for Predicting the Environmental Impact of Pesticides (SyPEP) (Beernarts and pussemier, 1997).

The EIQ system model was developed to help choose pesticides which are environmentally sound by Kovach *et al*. (1992), in assessing compatibility of pesticides with integrated pest management (IPM) practices to estimate the hazards to farm workers, consumers and ecological factors.

2.8 Vegetables Grown and their Yields in Kenya

In 2005 kales occupied a higher production area than other vegetables grown in Kenya (MOA, 2005). Cabbage and kale production almost exclusively target domestic markets hence their export share is less than 1%. French bean is the major export vegetable and account for 61% of the volume of vegetables exported in 2005 (MOA, 2005). A study done in 2005 showed that pesticide use in production of vegetables is quite high, with 62 formulations from 36 active ingredients. Table 2. 1 shows the major vegetables grown (PCPB, 2005).

Table 2. 2 Major vegetables grown in the subsector

Vegetable	N	Area (ha/farmer)[a]	Total area (ha)[b]	Production (tons)[b]	Value (million us$)[b]	Yield (t/ha)[c]
Kales	52	0.07(0.01)	26,818	315,159	21	12
Tomatoes	30	0.12(0.02)	20,743	337,447	44	16
Cabbage	295	0.22(0.02)	20,527	529,003	59	26
French beans	226	0.10(0.01)	7,004	62,189	17	9
Onions	7	0.07(0.02)	6,395	6,886	19	11
Garden peas	68	0.09(0.01)	5,313	22,798	6	4
Total	839	0.14(0.01)	86,800	1,266,596	166	13

Source: a= own data b= MOA (2005); N=number of surveyed famers producing the crops; Figures in brackets are standard errors.

A total of 62 pesticides products, comprising of 36 active ingredients, were in use in the subsector (MOA, 2005). Approximately, 52% were insecticides, 41% fungicides, 5% acaricides and 2% were herbicides. Based on the survey information and the reported vegetable/hectare in the MOA reports (MOA, 2005), approximately 263 tons of pesticides were used representing only 5% of the total amount of pesticide imported in 2005 (PCPB, 2005). The WHO has classified 57% by volume of pesticide applied as moderately hazardous, 8% as highly hazardous compounds, 25 % as carcinogenic and 43% as possible carcinogens (WHO, 2006). The application rate of 0.82 kg/ha of pesticides is fairly low compared to other countries in Latin America (7.17 kg/ha) and Asia (3.12 kg/ha) (Repetto and Baliga, 1996). However, some pesticides are extremely harmful even when used at low rates. The EIQ values calculated indicate that the sub-sector potentially has pesticide negative environmental impacts. Therefore a combination of pesticide and programmes to raise farmers’ awareness of pesticides that pose little threat would help safeguard the environment and human health.

2.9 Pesticide Residues in Food

When pesticides are used according to authorized agricultural practices, there are levels of residues expected to be in food that must not exceed the Maximum Residue Levels (MRLs) guidelines. These levels are always set far below levels that are safe for humans (EFSA, 2010). As from September 2008, the MRLS were harmonized in the EU under regulation EC 396/2005 (OECD, 2010). The presence of pesticide residues in food poses a potential risk to human health including the development of chronic diseases (Souza and Caldas, 2006).

Organochlorine pesticides are the pesticides that are extensively used by farmers compared to other types of pesticides. This is because they are cheap and they have broad spectrum of activity (Bempah *et al.*, 2011). However, their residues still appear in food as pollutant and also in the environment due to high level of persistence (Bempah *et al.,* 2011). As a consequence, incidences of crops produce having high levels of OC pesticides have been recorded (Bempah *et al.,* 2011). The challenge is that some of these pesticides are known carcinogens and may have long-term effects like nervous system disorder and immune suppression (Bempah *et al.,* 2011). Unpublished report from KEPHIS on a market basket and farm gate survey of tomatoes conducted in 1994 indicated levels above maximum residue limit (MRLs) for dithiocarbamates specifically Mancozeb (KEPHIS, 2006).

In 2005, Kenyan passion fruit and French beans were intercepted in Sweden and Belgium during routine monitoring (KEPHIS, 2006). The passion fruits were found to have dithiocarbamates and Chlorothalonil residues, while the French beans were found to have chlorothalonil, dimethoate, tebuconazole and omethoate residues (KEPHIS, 2006).

A study on dissipation and degradation of malathion and dimethoate from soil and garden pea carried out by Getanga (Getanga *at al.*, 2000) found the dissipation half-life of Dimethoate from the foliar surface of the garden pea to be 29 days ($t_{1/2}$ =29), while the foliar dissipation rate of malathion from the pea plant showed a fast decline during the initial stages and relatively slow afterwards.

A study carried out by Musila (2011) found that chlorothalonil has a dissipation half-life of 2 days in snow peas and 10.2 days in the soil. At pre-harvest interval of 21 days chlorothalonil residues measured were 0.01mg/kg for snow peas grown on untreated soil and 0.02 mg/kg for those grown on treated soils. Both levels were below both EU and Codex MRLs of 5 and 2 mg/kg respectively and the ADI of 0.03 mg/kg for snow peas (Musila, 2011).

In the Republic of Macedonia, a study was carried out on pesticide residues in plant- based foods which showed that 98% of the samples contained residues well below the relevant MRLs (Vesna *et al.,* 2014). This investigation showed that cucumber contained the highest number of pesticide residues with a predominance of methomyl (Vesna *et al.,* 2014). The other pesticide residues found in cucumber samples were boscalid, chlorpyrifos, cyprodimil, fenhexamid, imidacloprid, metalaxyl and tebuconazole (Vesna *et al.,* 2014). Table grapes showed a predominance of boscolid residues, but all were below the MRL (Vesna *et al.,* 2014). 1.19% of the total samples (Only two samples) had one pesticide residue which was above the MRL (Methomyl in cucumber, and chlorpyrifos in carrots) (Vesna *et al.,* 2014). In all samples of processed vegetables and fruits, there were no pesticide residues detected in all of them (Vesna *et al.,* 2014).

The main exposure route for pesticides is generally food. This exposure through the diet is assumed to be five times higher than other exposure routes like air and drinking water (Juraske *et al.,* 2009). It is expected that vegetables and fruits contain higher levels of pesticide residues than other groups of food that originate from plants such as bread and other food stuff that are consumed after serial processing (Claeys *et al.,* 2011) since vegetables and fruits are consumed when either semi-processed or raw. The use of pesticides in vegetables and fruits production is therefore constantly monitored due to the potential risk posed by pesticides to public health (Claeys *et al.,* 2011).

In Kumasi, Ghana, investigation carried out on pesticide contamination found synthetic pyrethroid and organochlorine pesticide residues in various vegetables and fruits either at the same or lower concentrations than the EU maximum residue limits (MRLs) (Bempah and Donkor, 2010) . They attributed low levels of pesticide residues to high water content but low lipids in the samples (Bempah and Donkor, 2010).

In Brazil, dithiocarbamates and ten organophosphates were analyzed and found in samples of prepared food collected at a University restaurant. Residues of dithiocarbamates found in samples ranged from levels below 0.10 to 0.24 mg/kg CS_2, with vegetables being the dominant group with detectable residues (Souza and Caldas, 2006).

In the Philippines, a pesticide residue monitoring study of commonly consumed fruits, vegetables and cereal including tomatoes, eggplants, potatoes, bananas, cabbages, carrots, mangoes, okra, rice and string beans found that 18.5% of the samples collected had detectable residues, with a small percentage (0.4%) having concentrations above the Japan MRLs (Chen *et al.,* 2006)

In Kenya, a survey of deltamethrin and lambdacyhalothin in vegetables reported mean residual levels ranging between 0.01 and 0.34 mg/kg during dry seasons and non-detectable levels during the wet season (Njagi, 2005). Vegetables consumed in urban areas had higher residues (0.34 mg/kg) compared to rural areas (<0.01 and 0.11 mg/kg). The levels were below MRL (maximum residual limit), however they were well above the ADI (Allowed Dairy intake) (Njagi, 2005).

In Ireland a monitoring programme was conducted by the Department of Agriculture and food in 2004 for crops to establish MRLs. The three different food crops were cereals, food of animal origin (milk, meat and dairy produce) and food of plant origin (vegetables and fruits) (DAFPCS, 2006). 29% of the samples were imports from outside the EU, 27% were taken from domestic market and 44% were from imports from other EU Countries. These samples were analysed for 118 pesticide and metabolites. Those samples that had no detectable pesticides residues were 48%, while those that contained one or more detected residues were 52% of the total number of sample of which 3.4% had residues above MRLs. In all, 53 residues of different pesticides were detected.

In the monitoring programme, Kenyan produce was found to have residues. In particular, Avocado was found to contain fludioxil at 0.02 mg/kg, while passion fruit samples were found to have propargite residues at 1.08 mg/kg and chlorothalonil at 1.15 mg/kg. While there were no MRLs for propargite the levels exceeded the EU MRL for chlorothalonil (0.01). Four samples of peas with pods had detectable residues. One contained dimethoate at 0.13 mg/kg which was above the MRL of 0.02 mg/kg. The second was found to contain dimethoate and tebuconazole at 0.12 and 0.05 mg/kg, respectively, and although there is no MRL for tebuconazole, the dimethoate MRL (0.02 mg/kg) was exceeded. The third sample contained 0.02 mg/kg dimethoate and 0.1 mg/kg omethoate but both were below the codex and EU MRL (1 mg/kg) for sugar peas. The fourth sample contained residues of kresoxim-methyl at 0.12 mg/kg which fell above the MRL (0.05 mg/kg). 13 cereal samples analysed had pesticide residues but they did not exceed the MRLs. Kenya, however, does not export cereals to the EU. Foods of animal origin including bovine, ovine, porcine, daily products, venison, poutly, eggs and honey samples were also in the 2004 PRM programme. 127 samples of bovine meat were analysed (DAFPCs, 2006).

The data concerning the dissipation rate and also on the pre-harvest intervals of Lufenuron, Bifenthrin and Iprodione pesticide in chili peppers, beans and peas is limited in the literature. For Bifenthrin, its dissipation rates have been reported in tea (Chen *et al.,* 2012). Only one study documents for Iprodione in greenhouse tomatoes (Omirou *et al.,* 2009), Lufenuron has been reported in spring onions and green beans (Hanafi *et al*., 2010).

The best techniques that have been considered to determine Bifenthrin in different sample matrices is chromatography. The two Gas chromatographic techniques used are, Gas chromatography using electron capture detector (GC-ECD) or Gas chromatography using flame ion detector (GC-FID). Liquid chromatography-mass spectrometry (LC-MS) is used in a few methods (Filho *et al.,* 2010; Botisi *et al.,* 2011).

Whereas cucumber is one of the most significant vegetable crops in Serbia, it is mostly affected by the downy mildew disease, forcing application of chlorothalonil and Metalaxyl-M for disease control (MacLachlan and Hamilton, 2010).

In the Serbian republic the PHI (pre-harvest interval) for metalaxyl-M and chlorothalonil is 7 days (MacLachlan and Hamilton, 2010). A study conducted to assess levels of these pesticides on crops established that after that period the levels of Metalaxyl M and chlorothalonil were under the Serbia MRLs of 0.5 mg/kg for Metalaxyl M and 1.0 mg/kg for chlorothalonil.

2.10 Preharvest Intervals

The values of pre-harvest interval (PHI) are the intervals when the residue levels are below the established MRLs and they are strongly influenced by prevailing environmental conditions (MacLachlan and Hamilton, 2010). In Egyptian farms, gray mildew, gray and white mold, and caterpillar in green beans, peas and chili peppers are controlled using Iprodione, Lufenuron and Bifenthrin. The MRLs for Iprodione are 5 mg/kg for beans and pepper, and 0.02 mg/kg for Lufenuron for green beans. The MRLs established for Bifenthrin are 0.2 mg/kg for pepper, 0.5 mg/kg for green bean and 0.1mg/kg for peas (EU, 2005).

2.11 Snow Peas Growing

The snow peas are a subspecies of *Pisum sativum* or it’s a distinct botanical cultivar also known as *Pisum sativum* macrocarpon. Its pod is slab-sided and it is eaten before the peas start to swell and string develops (Slinkard *et al.*, 1994). The crop is grown in higher altitude with cool and relatively humid climate preferably in the tropics with temperatures ranging from 7 to 30^{o}C (Slinkard *et al.*, 1994). Temperatures 16 $^{\circ}$C and 21 $^{\circ}$C during the day and 10 $^{\circ}$C and 16^{o}C during the night were reported by Slinkard *et al*. (1994) as the optimum temperature for the reproductive and vegetative periods of peas. The production of snow peas is concentrated between 50^{o} N and Tropic of Cancer. Peas can tolerate frost to -2^{o}C at the seedling stage but top growth may be affected at -6 $^{\circ}$C. On the other hand, winter hardy peas are able to withstand -10 $^{\circ}$C because of the snow cover protection, but the tolerance can also be as high as -40 $^{\circ}$C (Slinkard *et al.*, 1994). Temperatures above 27 $^{\circ}$C adversely affect pollination because they shorten the growing period. Hot spells on the other hand damage the peas more compared to a light frost. Peas can be grown where irrigation is practiced or during mid- summer and early fall in those areas having good rainfall and relatively low temperatures (Duke, 1981). A well-drained clay loam or silt loam is preferred for large yields; however, a sandy loam is preferred for very early crops (Duke, 1981). The most costly and critical part in snow peas farming is the harvesting period. In Kenya, snow peas production is a relatively recent introduction which is growing in importance due to its demand and value for the export market, mainly in Europe. Snow peas do well in the upper and lower highlands zones at altitude between 1,500 m and 2,600 m (Duke, 1981). Snow peas prefer cool temperature between 12 and 20 °C for cultivation and humid areas with well distributed rainfall of 1,500 mm to 2,100 mm per annum (Duke, 1981). Well drained soils rich in organic matter are suitable for snow peas production, whereas the silty clay loam soils are ideal and the optimum is soil with pH range 5- 7. In Kenya, the suitable areas for snow peas production include Nyandarua County, Kiambu, Meru (Timau area), upper Murang'a, Nyeri, Taita Taveta, Mt. Elgon, Bomet, Machakos, Koimbatek (Timboroa), Kericho and upper Kisii sub-counties. Some of the major diseases and insects that attack snow peas in Kenya are shown in Appendix VII (Table A6)

2.12 Irish Potato Growing

Irish potato (*Solanum tuberosum*) is one of the most common vegetables grown by farmers in Kenya (YAEI, 2012). They have a wide variety of uses, for instance they can be consumed when fried, boiled, roasted or mashed (YAEI, 2012). They are a good source of nutrition in that they are rich in carbohydrates, potassium, fiber and Vitamin C (Muraguri *et al*., 2012).

Potatoes also contribute to the Kenya's Gross Domestic Product (GDP). In 2012, the yield was 2.9 million tonnes which had a value of 50 billion Kenya shillings (HCDA, 2014). About 790,000 small holder farmers in Kenya produce the crop on a production area of 123,000 hectares (HCDA, 2014). This means that potato farming is a major employer and a source of income to the farmers and also to the industries which use it as a raw material for their production. The main potato growing areas in Kenya are Nyandarua, Nakuru, Meru and Bungoma counties (HCDA, 2014).

Even though this was a somewhat impressive yield, there is still potential for improvement once the various challenges faced by the farmers are addressed. Lack of quality seeds is one of these challenges. Farmers recycle their seeds resulting in poor yields (Muraguri, 2012). Poor crop rotation is another problem in potato farming. Farmers do not rotate the potatoes with other crops and this causes pest and disease build-up which affects yield and ultimately the income obtained (HCDA, 2014). Other constraints faced by farmers are seasonal variations in prices which cause variations in income, lack of finances for purchase of inputs, poor infrastructure in their areas in form of roads and telecommunication systems, lack of information on markets and new research varieties (Okello *et al.*, 2014). Potato crop is also affected by pests such as cyst nematode, potato tuber moth and leafminer fly (Okello *et al.*, 2014). Some of the diseases and insects that attack potatoes are shown in Appendix VII (Table A6).

CHAPTER THREE

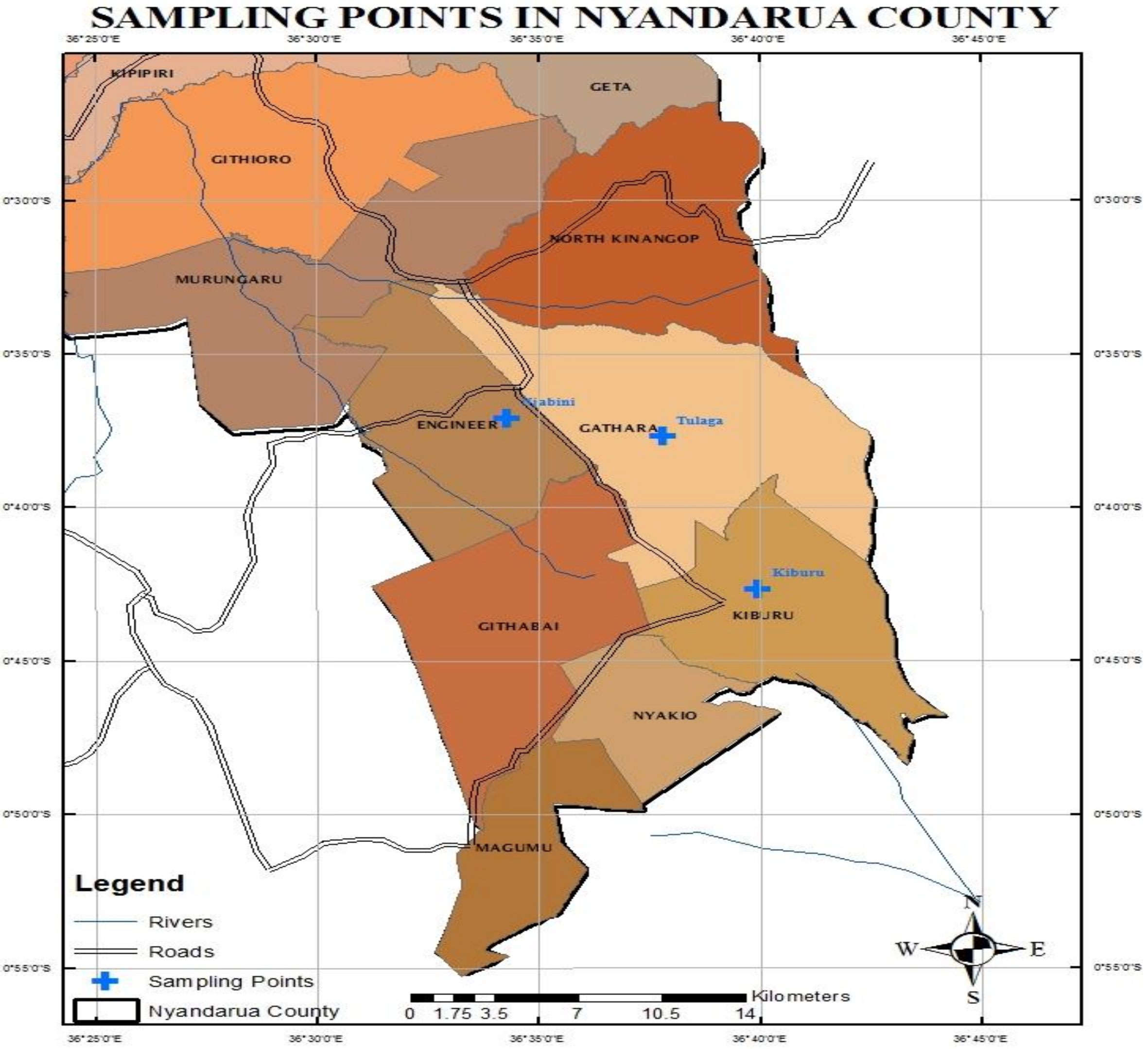
3. MATERIALS AND METHODS

3.1 The Design of the Study

The study had three parts: The first part involved administration of field questionnaires to identify the pesticides used and their social-economic impacts on the communities in the area studied. The second part involved planting potatoes and snow peas in an experimental garden, applying Ridomil Gold on potatoes and Actara 25 WG on snow peas and monitoring the Metalaxyl M and Thiamethoxam residue levels in soil and crops over a period of 28 days. The dissipation period was day 0, 2,4,7,14,21 and 28 for both pesticides. The third part was to determine the residue levels of Metalaxyl M in potatoes and Actara 25 WG in snow peas from thee different markets in Engineer, Nyandarua County.

3.1.1 Field Survey

The survey was conducted in three areas where farmers, chemical dealers and extension officers were interviewed on the use, safety, handling and environmental awareness of pesticides used in the farms. A total of 100 questionnaires were distributed as follows: farmers-80, chemical dealers-15, extension officers-5. A sample questionnaire that was used to collect information from farmers, chemical dealers and extension officers is presented in Appendix II. The results obtained were tabulated (Appendix III table A2). Figure 3.1 shows the map of study area.



Source: Cartographer, Department of Geology (2017)

Figure 3. 1 Map representing the study area (Kinangop, Nyandarua County).

3.2 Dissipation Studies and Experiments

The method of analysis involved taking samples from the experimental plots where these two crops were grown from the time of spraying to the time of harvesting. The dimensions of the experimental plot was 10m by 30m. It was sub-divided into three equal portions of 10m by 10m each. In the first portion potatoes were planted and the leaves were sprayed with Ridomil Gold using knapsack sprayer at the age of 21 days. Control pots were not sprayed. The amount of the active component of Ridomil Gold was analyzed periodically from day 0, 2, 4, 7, 14, 21 and 28 on both treated and untreated potato leaves. Leaves of potatoes were analyzed to ascertain the amount of active component of the Ridomil Gold absorbed at weekly intervals until potatoes were harvested. During this time the effect of fungi on leaves was observed.

These two parameters were compared and conclusion drawn on the effectiveness of using Ridomil Gold to control fungi on potatoes. In the second plot snow peas were planted and leaves were sprayed with Actara 25WG at the age of 21 days. Control pots were not sprayed. The amount of the active component of Actara 25WG was analyzed periodically from day 0, 2, 4, 7, 14, 21 and 28 on both treated and untreated snow peas leaves. Leaves of snow peas were analyzed to ascertain the amount of active component. The amount of the residual of Actara 25 WG on the final produce was compared with the recommended amount in the European market. A conclusion was drawn on whether the final produce was fit for export. The third plot was prepared and was sub-divided into two equal portion of 5m by 10m. In one portion the soil was sprayed with Ridomil Gold and the other with Actara 25WG using knapsack sprayer. No plant was planted in this third portion and a small part of it was not splayed with pesticide (untreated soil). The amount of the active component of the two pesticides was analyzed periodically from day 0, 2, 4, 7, 14, 21 and 28 on both treated and untreated soil.

3.3 Residue Analysis

The third part which involved investigating levels of selected pesticides in potatoes and snow peas in the market was carried out in the same area by collecting the samples from the selected plots and testing the levels of pesticide residuals (both Ridomil gold and Actara 25WG) in potatoes and snow peas, respectively. The samples were chemically dried using anhydrous sodium sulphate and extracted by Soxhlet method. Samples were cleaned and analysed by GC-MS for Metalaxyl M and GC-FID for Thiamethoxam. Analysis was carried out at the University of Nairobi, Chemistry Department, pesticides research laboratory.

3.4 Preparation of Drying Agents, Column Materials and Solvents

Anhydrous sodium sulphate, Na_2SO_4 was prepared by baking it for 16 hours at 200 $^{\circ}$C in the oven to remove all the impurities. SiO_2 (1.8 g per sample) were dried overnight at 200 $^{\circ}$C to make them 100% active (remove all water).

Deactivation of SiO_2 with water was done at 1.5%, w/w of water by adding 1.5 ml of HPLC grade water to 98.5 g of activated SiO_2 in a 250 ml Erlenmeyer flask and shaken by hand until all lumps were eliminated. The Erlenmeyer flask was placed on a shaking platform for half an hour. After deactivation, these chemicals were left overnight to condition. The performance of SiO_2 was tested before use with real samples.

General purpose grade acetone and hexane were each triple distilled before using them in the experiments. Copper powder was activated by shaking with 9 ml of dilute hydrochloric acid solution (3:1 ratio for H_2O: HCl). The mixture was centrifuged for 1 minute at 300 rpm and acid solution discarded. The powder was rinsed three times with methanol to eliminate all the hydrochloric acid, and the supernatant discarded. A gentle stream of nitrogen was used to dry the remaining powder before use.

3.5 Sample Processing

The laboratory samples of leaves, stems, roots and tuber in case of potatoes and unshelled pods in case of snow peas were homogenized using Hobart food processor. The homogenized wet analytical sample (10 g) was placed in a motor. For every gram of wet sample, 3 g of baked out anhydrous Na_2SO_4 was added. This mixture was ground using a pestle to a homogeneous powder. This was then covered with aluminium foil and left overnight to dry further. This was done in triplicate.

The dry sample for extraction was put in a thimble and placed in a Soxhlet. Hexane and acetone in the ratio of 1:1 was placed in a 250 ml round bottomed flask and the Soxhlet extraction was set in triplicate for each sample and was allowed to extract the sample for 16 hours.

Three replicates (5 g each) of the homogenized wet sample were weighed in a weighing glass and kept in an oven to dry for moisture content determination. After drying, the dry mass was re-weighed and the moisture content determined from the two masses. This was repeated for all the samples extracted.

Soil extraction procedure was adopted from UNEP POP Training Manual 2010. 20 g of each soil sample was weighed into a beaker and baked out Na_2SO_4 added in the ratio of 1 g of sample to 2 g of Na_2SO_4. The contents were stirred to obtain a dry mixture, covered with aluminium foil and left overnight to dry further. Soxhlet extraction was set as described above and allowed to extract for 16 hours. In all cases anti-bumping chips were added to allow smooth boiling. The extract was concentrated using rotary evaporator to 1ml and then taken for clean-up.

3.6 Sample Extracts Clean-up

Sample clean-up was done using silica. A chromatographic clean-up column 20 cm long and 2 cm internal diameter (with frit) was packed with anhydrous Na_2SO_4 to 1cm height (equivalent to 2 g) and 1.8 g of SiO_2 followed by 0.5 g of activated charcoal and another 1 cm of Na_2SO_4. After every addition, the column was tapped to allow the particles to settle uniformly. The column was then conditioned by eluting with 15 ml of hexane: acetone mixture (ratio 1:1) and discarded.

The Soxhlet extract of the sample was transferred quantitatively to the column. The tube was further rinsed with five 1 ml portions of hexane: acetone mixture and the rinsing transferred to the column as soon as the sample extract had eluted without leaving the column to completely dry. 165 ml of hexane: acetone mixture (ratio 1:1) was eluted through the column. A 250 ml round bottomed flask was used to collect the extract. The eluent was rotary-evaporated to 1.5 ml and then transferred to a GC sample auto vial. A gentle stream of nitrogen was used to further concentrate the extract to 1 ml. The extract was now ready for analysis by GC-MS for Metalaxyl M and GC-FID for Thiamethoxam.

3.7 Sulphur Removal

To remove sulphur from the cleaned soil extracts, approximately 1 g of freshly activated copper powder was added to the sample in a vial. A black coloring appeared to all extracts containing sulphur which indicated a formation of copper sulphide compound. The extracts were then filtered through glass funnel which was packed with glass wool and activated 2 g anhydrous sodium sulphate. 5 ml of HPLC hexane: acetone mixture (ratio 1:1) was used to condition the anhydrous sodium sulphate and the sample was introduced then eluted with 20 ml of HPLC grade hexane: acetone mixture (1:1). The extract was collected in a round bottom flask and it was concentrated to 1 ml using rotary evaporator. The concentrated extract were then transferred to clean auto vials and then concentrated to 0.5 ml under a gentle stream of white spot nitrogen gas and taken for GC-MS for Metalaxyl M analysis and GC-FID for Thiamethoxam analysis.

3.8 Recovery Tests

Triplicates of 10 g plant and 20 g soil samples separately were each spiked with 50 μl of 1 ppm Metalaxyl M standard solution and dried with activated anhydrous 30 g Na_2SO_4 and blended to homogenous powder, before transferring to the Soxhlet thimble for extraction. The extraction procedures and clean-up process were performed as illustrated in sections 3.5 and 3.6, respectively.

The recovery tests was performed by blending 10 g of dried activated anhydrous Na_2SO_4 using mortar and pestle until homogenous powder was obtained (blank sample). The blank sample was spiked with 50 µl of 1ppm Metalaxyl M standard solution. The extraction, clean-up and analysis procedures were as described in sections 3.5 and 3.6, 3.7, respectively. The percentage recoveries were calculated. The whole procedure was repeated with Thiamethoxam standard solution.

3.9 GC-MS Analysis and Quantification of the Extract Samples

Analysis of the pesticide residues was carried out using GC-MS (mass spectrometer model HP5771A and the NIST library 2008) at the Department of Chemistry pesticide laboratory, University of Nairobi. The DB-5 fused silica capillary column of 30 m x 0.25 µm i.d. x 0.25 µm film thickness was coated with cross-linked 5% phenyl dimethyl polysiloxane. The carrier gas was helium (99.99 % purity) at a flow rate of 1.0ml/min. Oven temperature was maintained initially at 70 °C for 1 min, increased at 15 °C/min to 175 °C, then at 2 °C/min to 215 °C, at 10 °C/min to 265 °C and finally at 20 °C/min to 290 °C and held for 8 minutes. Injection volume was 1µL, injected in split-less mode at injection temperature of 250 °C.

3.10 Quality Assurance and Quality Control

Control pots for dissipation of metalaxyl M in potatoes and thiamethoxam in snow peas and both pesticides in soil were set alongside the experimental pots. The control pots were separated to exclude any contamination from the treated pots. Analysis of control samples including untreated potato and snow peas plants and untreated soil for both pesticides was carried out alongside the samples to ascertain that no artifact in the crop derived from local conditions gave rise to interference in the analysis and to establish the transport and storage stability of any residue. Field blanks consisting of distilled anhydrous Na_2SO_4 were carried along at every field trip to track field contamination and were then subjected to the entire analytical procedure as the samples. Extraction was carried out in triplicate.

3.11 Moisture Content Determination

Moisture content in plant and soil samples was determined by heating 5 g of the sample in pre-cleaned and pre-weighed watch glass in an oven (model E 28# 04- 71528) at 105 ºC for 24 hours. The difference in weight between wet and dry sample was taken as the moisture content. Percentage moisture was calculated as:

$$\%\text{moisture} = \frac{\text{moisture content}}{\text{Weight of wet sample}} \times 100 \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \;(1)$$

3.12 Statistical Data Analysis

Statistical programme for social scientists (SPSS) together with Microsoft Excel programme were used for the analysing data. Calculation of half-life was done using Langmuir rate equations.

$$R \;\alpha\; dC/dt = kKC/(1+KC) \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \;(2)$$

Where, R = rate of reaction (mol/L.min); C = equilibrium concentration of reagent (mol/ L); t = time (min); k = rate constant (1/min), and K = Langmuir constant (L/mol).

Calibration standard series used were evaluated for within laboratory reproducibility acceptability. Results are presented as mean of triplicate analysis with standard deviation.

3.13 Physicochemical Parameters of Soil

The soil was analysed for metalaxyl M, thiamethoxam and both physical and chemical parameters to obtain baseline information. The soil was taken to Kenya Agricultural Research Institute (KARI) to carry out physiochemical analysis. Methods of soil analysis as described in physical and chemical methods of soil analysis were used to carry out soil analysis (MoA, 1980). Soil analysis involved physical and chemical analyses. The physical analyses included texture analysis also called mechanical or particle size analysis by pipette method, complete moisture retention (pF) where undisturbed soil core samples were subjected to negative pressure suction (kaolin box apparatus) and positive external gas pressure (pressure cooker) to remove water, and bulk density determined according to the core method where the double cylinder core samplers were used. Porosity and particle specific density were also analysed. The double cylinder core samplers used were 5 cm diameter by 5 cm height.

The chemical analysis included pH determination in a 1:1 (w/v) soil-water suspension with a pH meter, total organic carbon using the calorimetric method, where all organic carbon in the soil sample was oxidised by a 15 ml mixture of concentrated sulphuric acid and 5% potassium dichromate in 1:2 ratio v/v at 150 $^{\circ}$C for thirty minutes to ensure complete oxidation. Barium chloride was added to the cool digest, mixed thoroughly and allowed to stand overnight. The amount of organic carbon in samples was determined by the concentration of chromic ions (Cr^{3+}) produced after oxidation spectrophotometrically at 600 nm.

Total nitrogen was analysed using the Kjeldhal method where organic nitrogen compounds in soil are digested with concentrated sulphuric acid and selenium mixture as a catalyst to convert nitrogen to ammonium sulphate. The digest was made alkaline with sodium hydroxide and the released ammonia distilled off and trapped in boric acid indicator solution and titrated against standard acid.

Other available nutrients like phosphorous, magnesium, manganese, calcium, potassium and sodium were analysed using Mehlic 1 (double acid) method. Oven dry soil was extracted in a mixture of 0.1 M hydrochloric acid and 0.5 M sulphuric acid; 1:5 ratios (w/v) where the acid replaces the bulk exchangeable metal cations and the sulphate anion is exchanged for phosphate. The P, Mg and Mn were determined calorimetrically, while Ca, K and Na were determined by flame photometry.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Pesticides Use Survey in Kinangop Sub-County

The survey was conducted in three sampling sites (Kiburu, Tulaga and Njabini) where 100 respondents were interviewed on the use, safety handling, effectiveness of pesticides used to control pests and diseases and environmental awareness of pesticides used in the farms. All the farmers interviewed were the owners of their farms and grow their plants on open field. All the chemical dealers operate their businesses in the shopping centres in these sampling sites. All the extension officers are employed by the Government to offer extension services to these farmers and are stationed in Sub-County headquarters. The summary of the survey is attached on the appendix III Table A2.

4.1.1 Background information on Farming in the Three Sampling Areas.

A total of 100 questionnaires were distributed as follows: farmers-80, chemical dealers-15, extension officers-5. A total of 22 (75.86%) questionnaires were returned from Kiburu, 31 (77.50%) from Njabini and 21 (67.74%) from Tulaga (Appendix III Table A2). The average percentage returned from the three sampling areas ((75.86+77.50+67.74)/3 = 73.70%. The 73.70 % return constituted adequate number of questionnaires for statistical analysis and for development of conclusions on pesticide management practices and awareness activities. Figure 4.1 below shows the distribution of questionnaires in the various sampling sites within Kinangop Sub County.

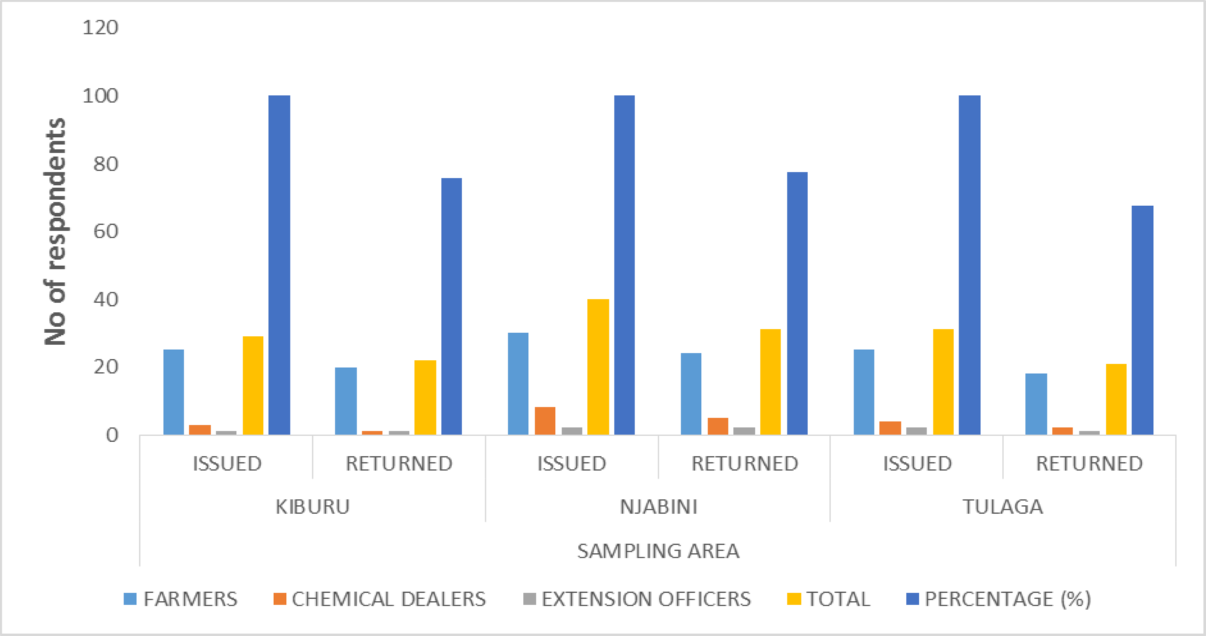


Figure 4.1 Percentage of Questionnaires returned

4.1.2 General information from Questionnaires Returned.

The survey indicated that 65% of farmers in Kinangop sub-county are men while 35% of farmers are women. In Kiburu and Njabini, there were more male respondents than female while in Tulaga there were more female respondents than male. This indicates that in both Kiburu and Njabini there may be more males who are involved in farming than female while in Tulaga more female may be involved in farming than male (Figure 4.2).

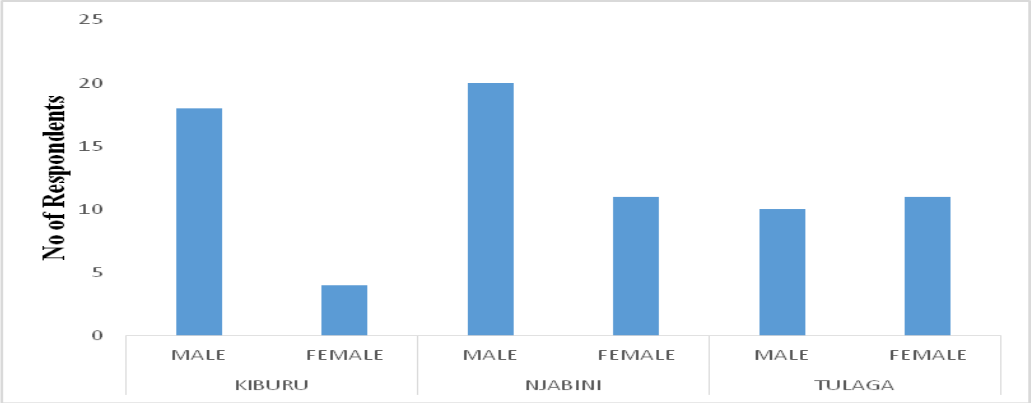


Figure 4.2 Gender of the respondents

4.1.3 Level of Education of the Respondents

20% of the respondents had primary education, 54% had secondary education while 26% had post secondary education. All the chemical dealers and extension officers had post-secondary level of education but very few farmers had post-secondary level of education. Kiburu had 5 respondents with primary level of education, Njabini 4 whereas Tulaga had 6. This shows that Tulaga had the highest number of respondents with low level of education while Njabini had the lowest number of farmers with low level of education. Those with secondary level of education in Kiburu were 12, Njabini 18 and Tulaga had 10. This indicates that Njabini had the highest number of respondents with secondary level of education and Tulaga had the lowest. The number of respondents with post-secondary level of education were as follows: Kiburu 5, Njabini 9 and Tulaga had 5. Njabini had the highest number of respondents with the highest level of education as shown in Figure 4.3 for total number of respondents per category of education, and Appendix III Table A2.

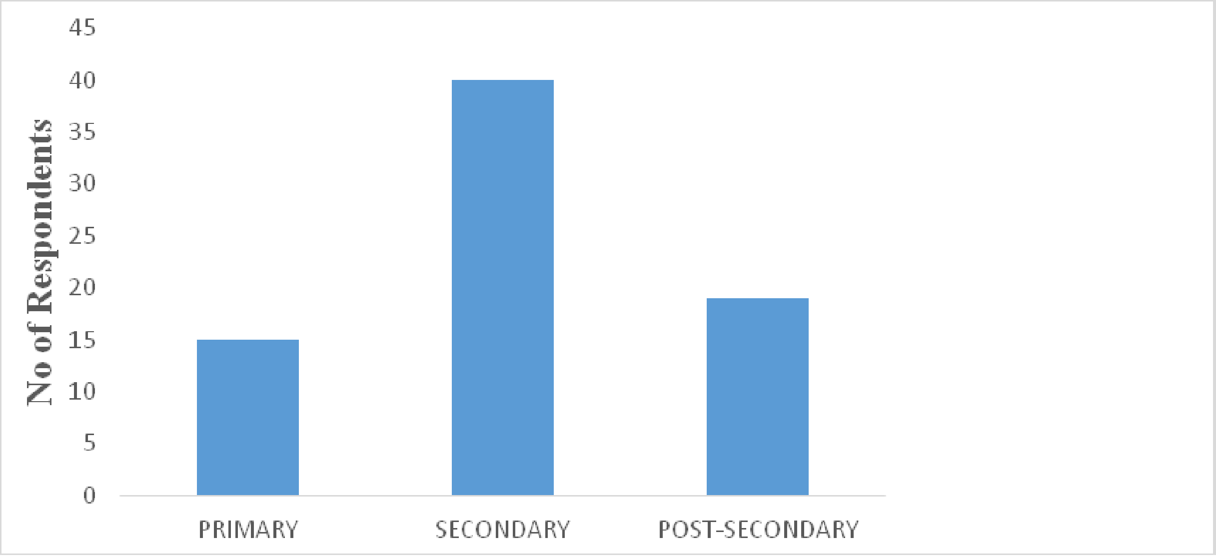


Figure 4. 3Level of education of the respondents

4.1.4 Age of the Respondents

60% of the farmers interviewed indicated that they were over 35 years old (Figure 4.4 and Appendix III Table A2). This indicated that majority of the young people were not involved in farming, probably due to the fact that agricultural farmers were associated with low income from the farming venture that discouraged the youth. However, the perspective could be reversed by using the morden methods of farming such as mechanising and reducing the threat caused by pest and diseases that reduce the farm produce.

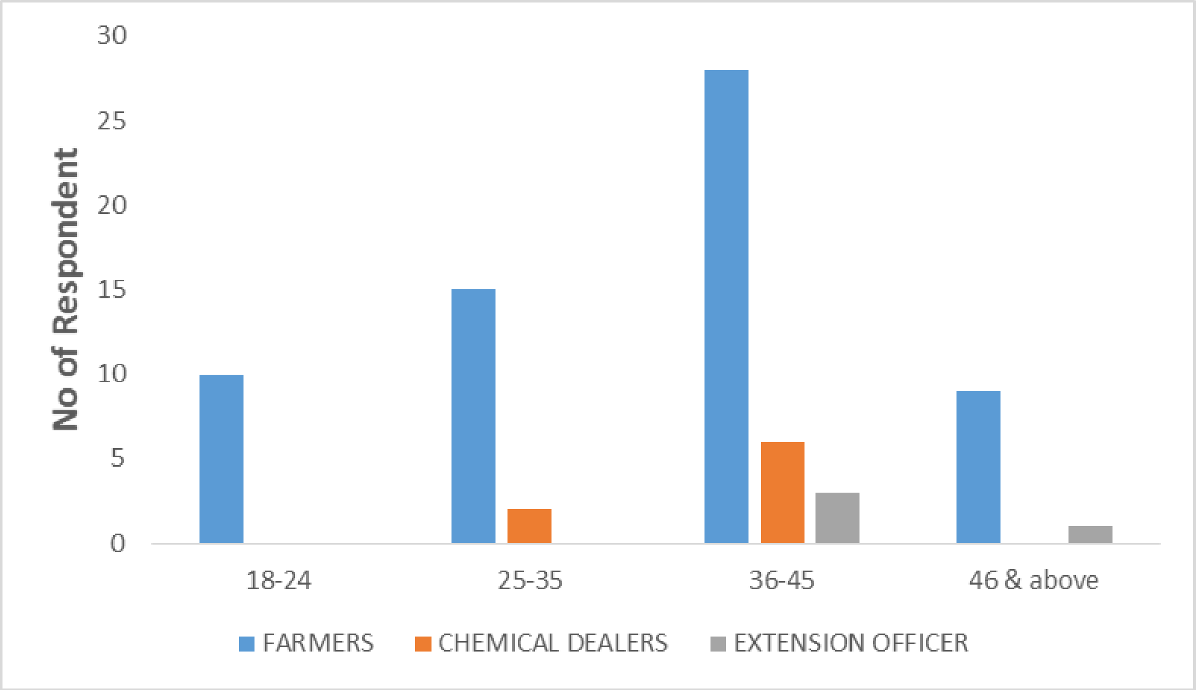


Figure 4. 4Age of Respondents

4.1.5 Pesticides Used in Kinangop Sub-County and their Classifications

The most commonly used pesticides in Kinangop Sub-County are organophosphates, carbamates and pyrethroids. Table 4.1 shows the major pesticides identified by the respondents. Further assessment of the identified pesticides against the official list of pesticides registered by the Pest Control Products Board in Kenya (PCPB, 2004) revealed that all the pesticides were officially registered in the country.

Table 4.1 Pesticides Used in Kinangop Sub-County and their Classifications

Pesticides	**Active ingredient (a.i)**	**Class**	**Toxic Classification**	**Used by% Household**	**Crops Treated**
Karate	λ-cyhalothrin	Synthetic Pyrethroid	WHO II	64	Vegetable, Tomatoes
Milraz	Mancozeb	Carbamate	WHOIII	78	Tomatoes
Dithane	Mancozeb	Carbamate	WHOIII	54	Tomatoes, vegetables
Dimethoate	Dimethoate	Organo phosphate	WHO II	32	FrenchBeans, Tomatoes, Vegetables
Dursban	Chlorpyrifos	Organo phosphate	WHOII	81	French beans, Beans
Ridomil	Mancozeb	Carbamate	WHOIII	15	Potatoes
Milthane	Mancozeb	Carbamate	WHO III	42	Vegetables
Furdan 5G	Carbofuran	Carbamate	WHOI	54	Vegetables
Diazinon	Diazinon	Organophosphate	WHOII	92	Animals, Fruits
Antracol	Propinab	Carbamate	WHOIII	39	Potatoes, Vegetables
Round Up	Glyphosate	Organo Phosphate	WHOII	65	Tomatoes, Maize
Sumithin	Fenithrothion	Organo Phosphate	WHO II	11	In door use
Ambush	Cypermethrin	Synthetic pyrethroid	WHO III	87	Vegetabes
Tactic	Amitraz	Organo phosphate	WHO III	45	Vegetables
Actara 25WG	Thiamethoxam	Carbamate	WHO III	51	Snow Peas, potatoes

Note: 1) Pesticide data obtained on 22/ March 2016; 2) WHO I to III classification based on (WHO, 2002). I is the most hazardous.

4.1.6 Factors Affecting Choice of Pesticide

The potential of pesticide to enable the farmers to get better yield was the main consideration for use of a particular pesticide as indicated by 48.4% of the respondents. Cost effectiveness of the pesticide followed with 32.3% of the respondents noting it as an important factor, whereas 4.8% of the respondents used a particular pesticide because it was suggested by the sales people from agrochemical industries or agrochemical dealers. 9.7% of the respondents who indicated that they used pesticides because they were suggested to them by other farmers, while 4.8% of the farmers used the pesticide because it was good for the environment (Figure 4.5 and Appendix III Table A2).

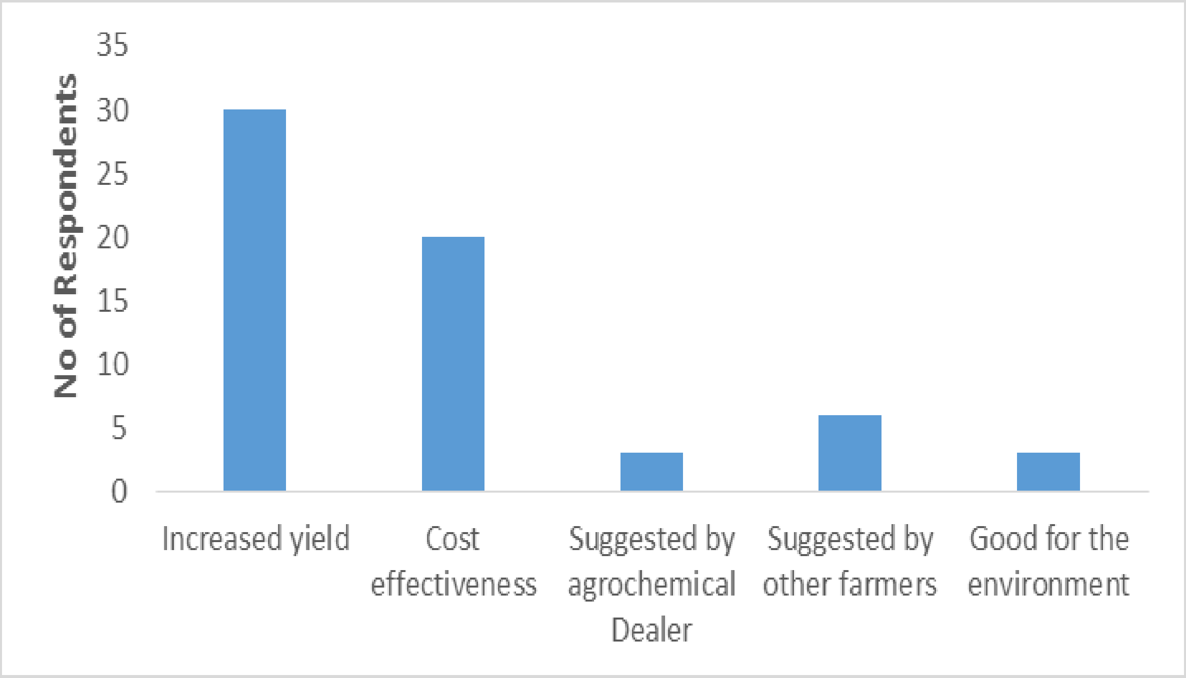


Figure 4.5 Factors affecting the choice of pesticide by farmers

4.1.7 Impacts of Pesticides on the Environment

It was noted that 72% of the respondents were aware that the use of some of the pesticides had been banned or restricted in Kenya, while 28% of the respondents had no information on the restrictions or ban of these pesticides. 89% of the respondents were aware that pesticides contaminate the environment in case of poisoning the environment with pesticides while 11 % were not aware (Appendix III Table A2). Figure 4.6 shows the level of awareness of farmers regarding pesticides effects on environment.

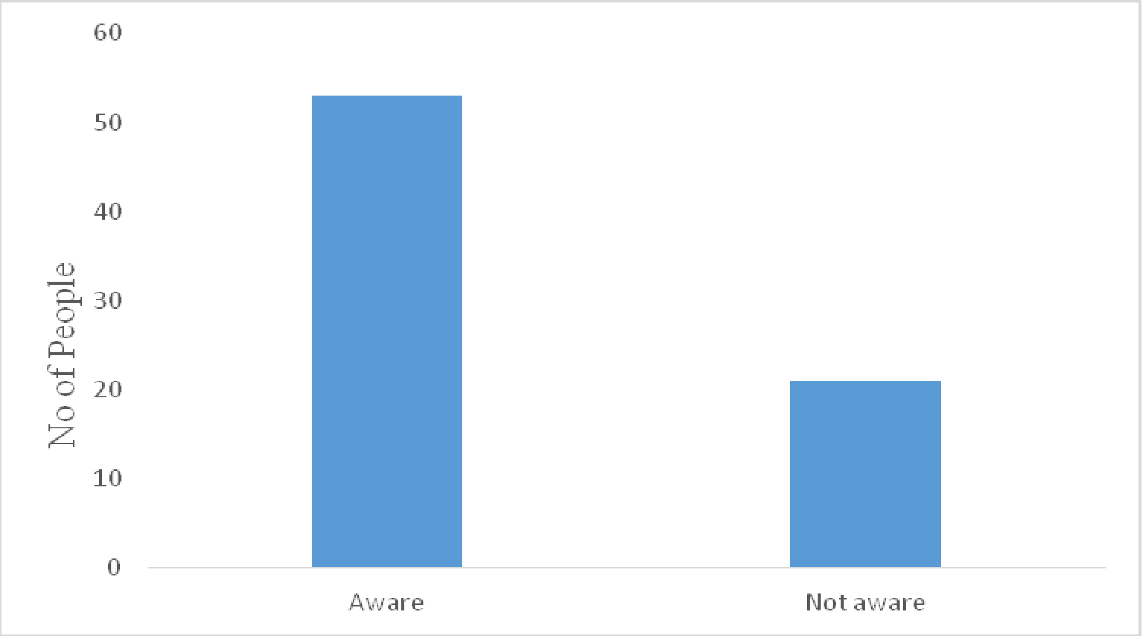


Figure 4. 6Effects of Pesticides to the Environment

4.1.8 Training on pesticide management & safety

All the respondents indicated that they were aware of the use of pesticides, but 54% had basic training on pesticide management, while 80% had basic training on the use of protection clothing when handling pesticides. 42% of the farmers even those who had training on how to handle pesticides and also knowledge of the effects of pesticides did not use protective devices when handling pesticides. They complained that the devices were expensive and hence they could not afford them. 72% of the respondents always read instruction on the pesticide products before use while 6% were not aware of the instruction menu. 22% did not read the instruction menu at all, which was attributed mostly to ignorance. The detailed results on responses obtained from the questionnaires are provided in Appendix III Table A2. Farmers who had higher level of education above secondary education always read instructions on the pesticide use and formulation, while majority of those with secondary education always read the instruction about the appropriate application of pesticides but they did not read on proper mixing of the pesticides. The respondents who had only primary level of education did not read the instructions at all. As shown in Figure 4.7.

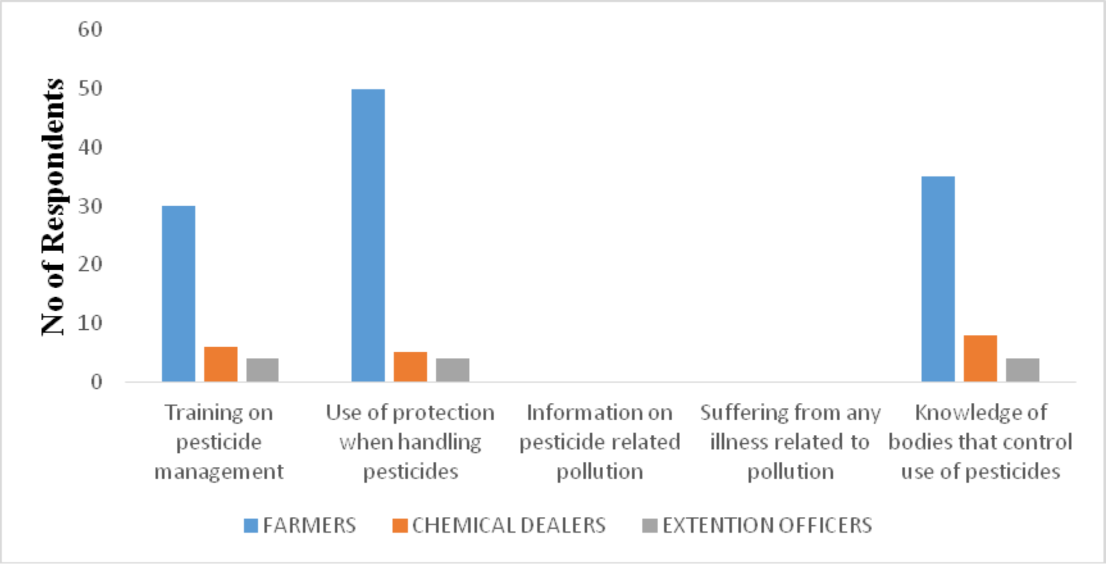


Figure 4. 7 Training on pesticide management & safety

4.1.9 Effectiveness of Pesticides.

Only 1.3 % of the respondents felt that pesticides are very good in controlling pest and diseases, 16.2 % felt that they are good, 20.3 % felt that they are fair whereas 62.2% felt that pesticides are poor in controlling pest and diseases (Appendix III Table A2). Many farmers felt that the pesticides were not effective because their crops were affected by pests and diseases even after applying pesticides. Chemical dealers on the other hand believed that the pesticides were effective and that the majority of the farmers were not following instructions given for application of a particular pesticide leading to either over dose or under dose. Extension workers also shared similar opinion with the dealers that due to financial constrains many farmers were aware of dosage rate but were unable to buy and apply required amount and follow the frequency recommended. Figure 4.8 below shows the responses from the respondents.

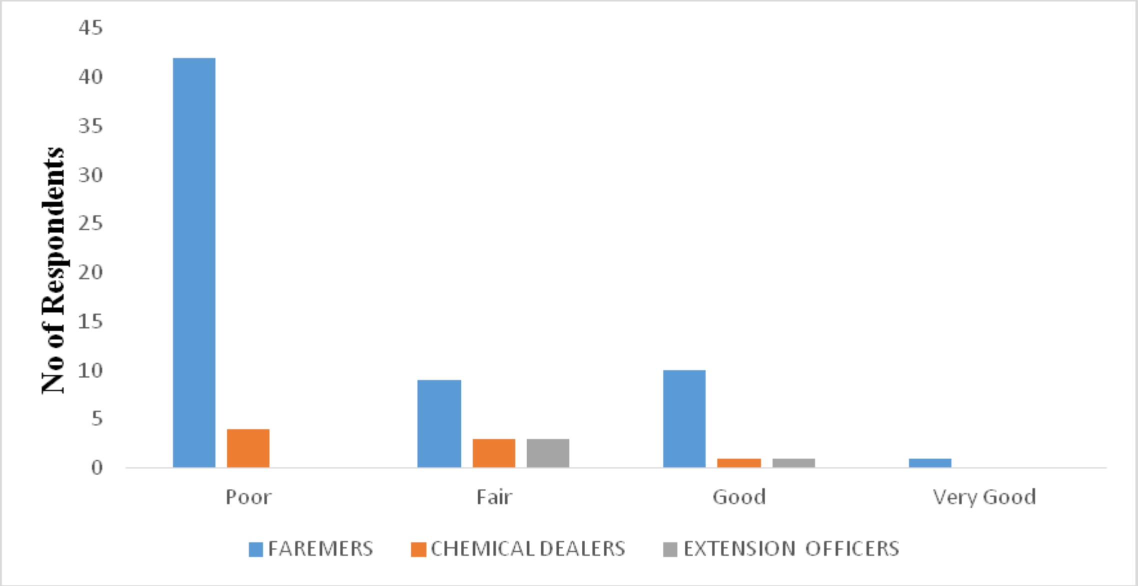


Figure 4.8 Rating of pesticides used.

4.1.10 Training sessions on use of pesticides for farmers

Based on the responses from the extension workers and the chemical dealers, it was noted that training sessions were not frequent, since most of them were conducted on annual basis, on demand or when a new product was being introduced into the market. Figure 4.9 below shows the responses on the frequency of pesticide trainings among different categories of stakeholders.

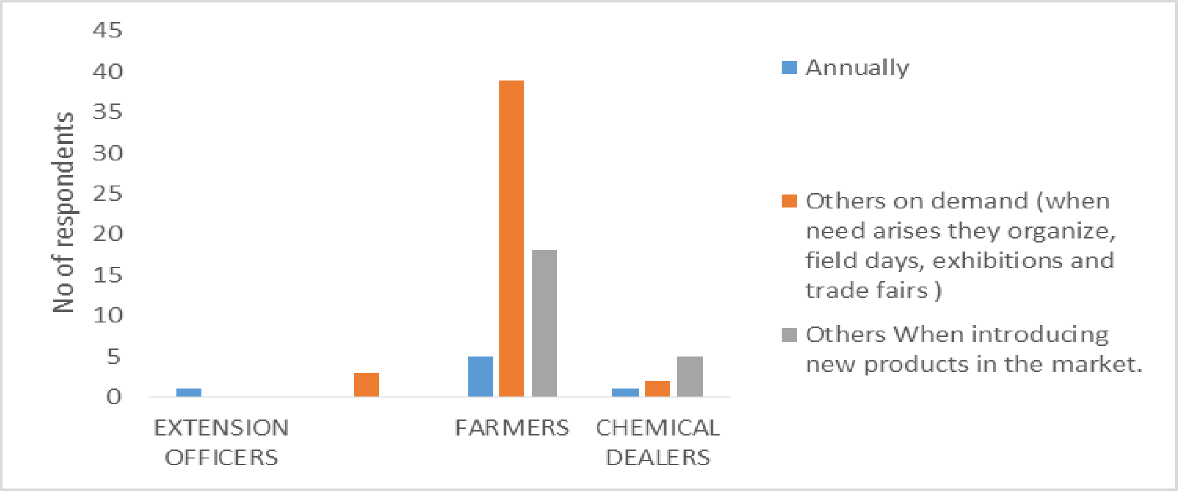


Figure 4.9 Training sessions on use of pesticides

4.1.11 Common Challenges of the Farmers

Farmers in the study area faced many challenges which included unpredictable weather patterns, fluctuating prices for their farm produce, lack of market for their produce and inadequate personal protective clothing for safe handling of pesticides. Other challenges stated by respondents were high cost of pesticides which sometimes lead to under dosing. Majority of farmers lacked knowledge on P.H.I and on how to measure granular and powder pesticides. How to store the remaining chemicals was also stated as a challenge faced by many farmers.

4.2 Physicochemical Characteristics of Soils from the Study Area

The soil in the experimental garden contained 77% clay, 7.1% sand and 15.9% silt. It was slightly acidic with a pH of 6.50 (Table A1, Appendix I). The percentage total nitrogen was 0.52 while the organic carbon was 3.62%. All macro elements potassium, calcium, magnesium and sodium were adequate except phosphorous which was low at 27.5 mg/kg based on the Mehlich double acid method (Mehlich 1) whose critical level for phosphorous is 30 mg/kg. The micro-elements copper, iron and manganese were adequate while zinc was low at 4.38 mg/kg. The saturation point of the soil at PF 0 was 80.48% while the field capacity point of the soil at PF 2.0 and 2.3 were 62.17% and 42.67%, respectively. The permanent wilting point of the soil at PF 4.2 was 20.45%. The soil had bulk density of 0.72 g/cm^3. Characteristics of the soil are summerised in Table A1, Appendix I.

4.3 Method Accuracy and Recovery Tests

Recovery data for pesticide residue analysis were calculated from blank soil samples fortified with 50 µl of 1 mg/L Metalaxyl M stock solution and 50 µl of 1 mg/L thiamethoxam stock solution. These were then analysed to establish the recovery of the analyte using different solvents. The cleanup stage was optimised and gave recovery of 105.30 % for metalaxyl M and 101.40 % for thiamethoxam with elution of 165 ml hexane:acetone (ratio 1:1), 95 % for metalaxyl M and 92 % for thiamethoxam with elution of 165 ml of hexane. 165 ml of hexane:acetone mixture (ratio 1:1) was therefore chosen as solvent for eluting pesticides during clean up since it gave the highest recovery.

Samples of potatoes, soil and blank (unhydrous Na_2SO_4) were spiked with 50 µl of 1 mg/L metalaxyl M and similaly samples of snow peas, soil and blank (unhydrous Na_2SO_4) were spiked with 50 µl of 1 mg/L. The percentage recoveries of thiamethoxam were calculated. The recoveries of metalaxyl M and thiamethoxam ranged between 75.15 % and 95.35 %.

The recovery of metalaxyl M was found to be 86.50 % in soil, 75.15 % in potatoes and 92.95 % in blank samples respectively. The recoveries of thiamethoxam were found to be 89.25% in soil, 80.65% in snow peas and 95.35% in blank samples, respectively. The recoveries were within the acceptable range of 70 % - 120 % (Hill, 2000). Detailed recovery data is provided in Table 4.2.

Table 4.2 Method Recovery, data in % for Metalaxyl M and Thiamethoxam Residues

Commodity	Metalaxyl m	Thiamethoxam
Potatoes	75.15±2.3	
Snow peas		80.65±5.6
Soil	86.50±2.8	89.25±8.4
Control	92.95±5.4	95.35±1.9

4.4 Quantification Tests

Quantification was based on external standard method based on concentrations from 2.46 mg/L to 126.30 mg/L and 1.95 mg/L to 172.08 mg/L for metalaxyl M and thiamethoxam, respectively. The calibration curves were drawn by plotting instrument response (peak area) against analyte concentration. The R^2 values were above 0.99 indicating good correlation between instrument responses and analytes concentrations. Figure 4.10 and 4.13 below show calibration curves for metalaxyl M and thiamethoxam, respectively. Sample analytes concentrations were determined following the regression equation $Y = mx + c$

Where, y = Peak area (Instrument response), x = Analyte concentration; m = gradient; and c = constant.

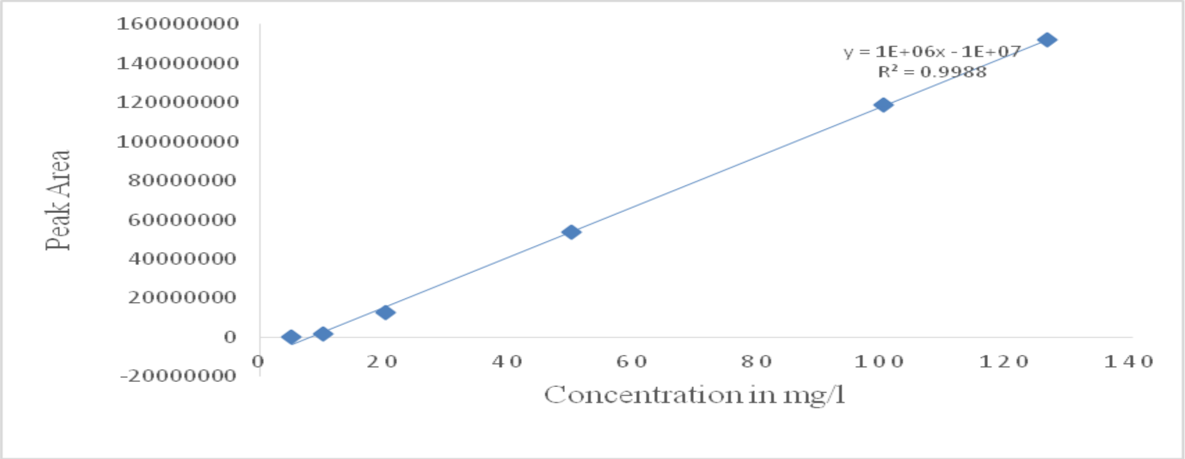


Figure 4. 10 Calibration curve of Metalaxyl M standard

Figure 4.11 below shows the ion chromatogram of metalaxyl M after analysis using gas chromatography equipped with mass spectrometer.

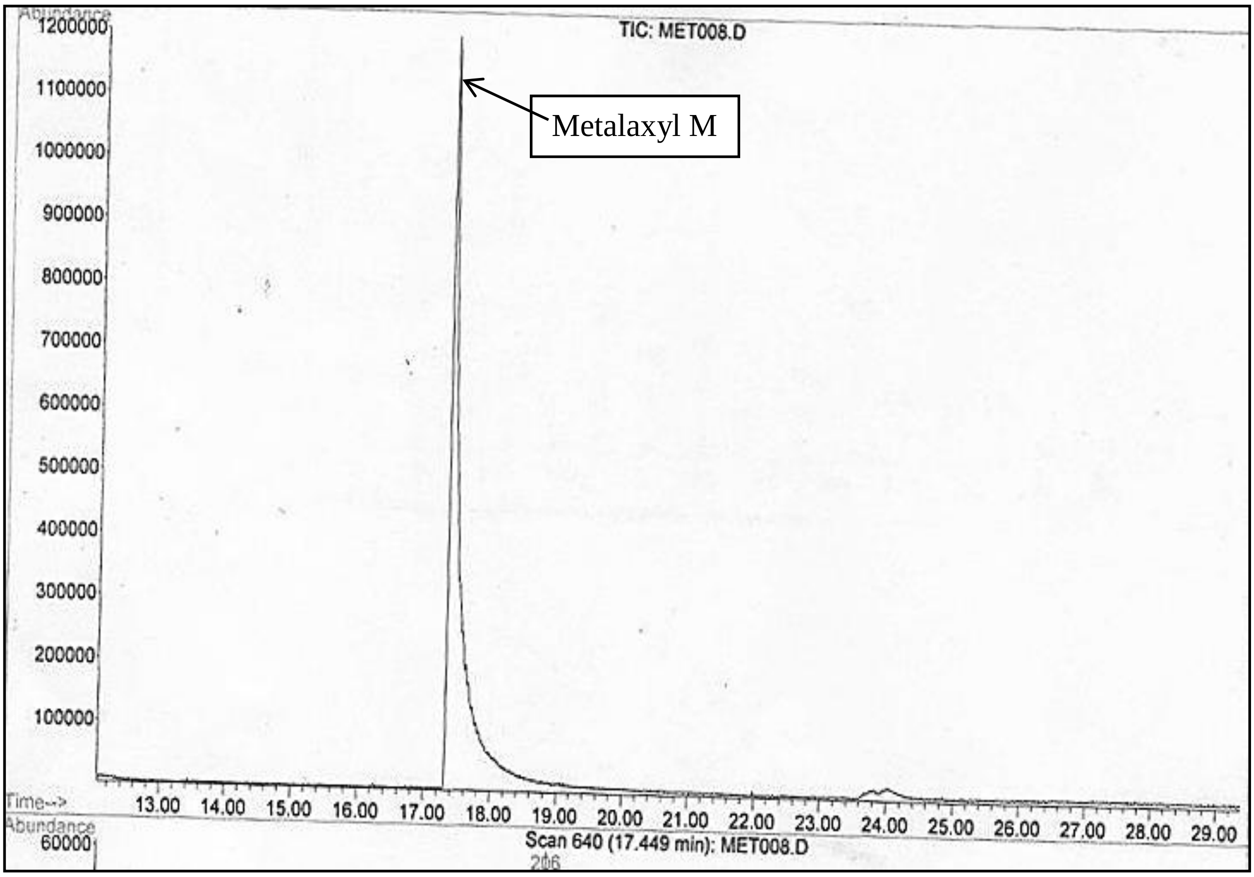


Figure 4.11 GC/MS ion chromatogram for Metalaxyl M standard

Identification of metalaxyl M was done using mass spectrometer model HP5771A and the NIST library 2008. Figure 4.12 below shows the mass spectrum of metalaxyl M standard solution when run on the gas chromatography-mass spectrometer.

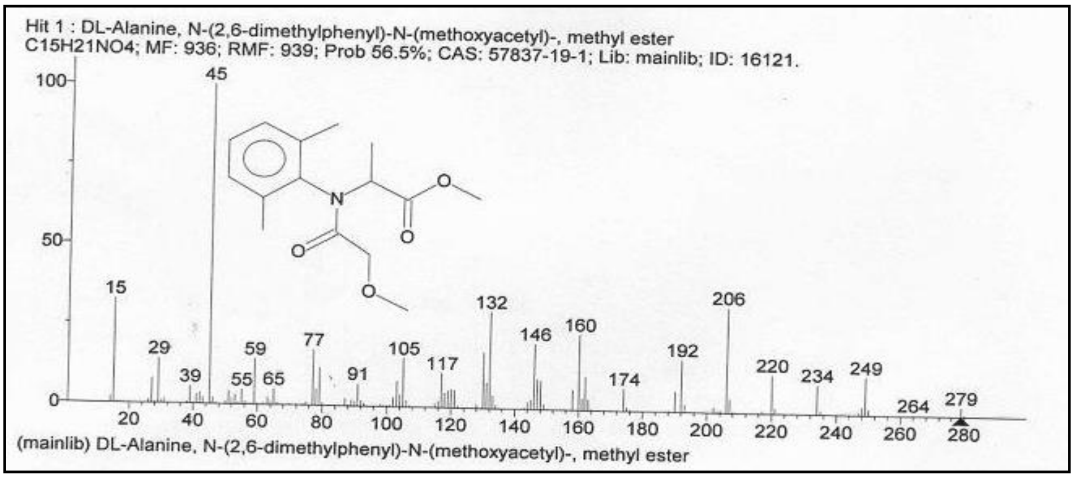


Figure 4. 12 Chemical structure and ionic mass Spectra for Metalaxyl M standard

4.5 Quantification of Thiamethoxam

Quantification of thiamethoxam was based on external standard method where standard solutions of different concentration which generated a calibration curve (Figure 4.13). Good linearity was obtained with $R^2 = 0.99$.

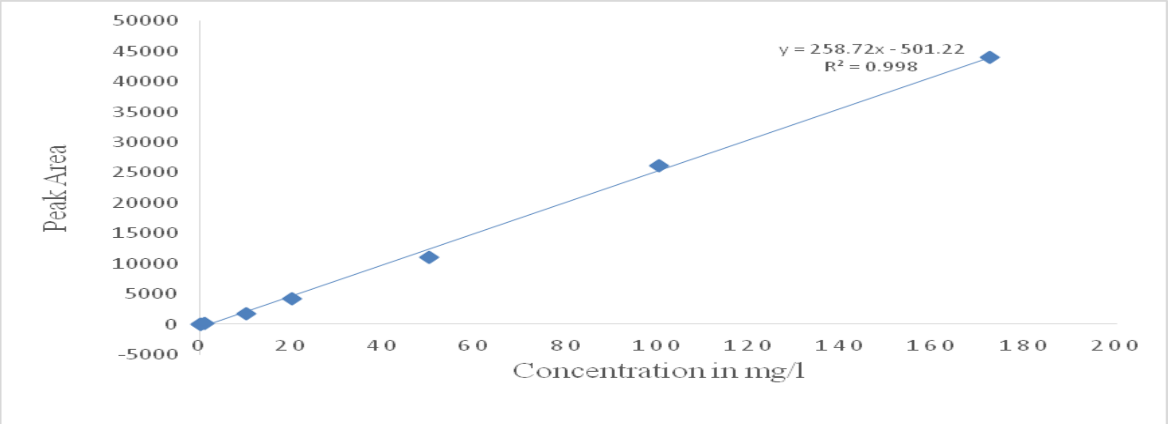


Figure 4. 13 Calibration curve of Thiamethoxam standard

The calibration curve gave an equation y = 258.72x-501 (R^2 = 0998) which was used to calculate the concentration of thiamethoxam.

4.6 Dissipation of Metalaxyl M in Potatoes leaves

The spray mixture applied to the potatoes contained Metalaxyl M 40 g/kg of Ridomil Gold. The plants growing on the soil had non-detectable residues of metalaxyl M. The potatoes that mature in 90 days were treated with metalaxyl M only once to determine the effectiveness of a single application. Samples were collected after a determined period of time from day 0, 2, 4, 7, 14, 21 and 28. The collected samples were extracted and analysed using GC/MS. The results obtained are presented in Table 4.3.

Table 4.3 Results of Metalaxyl M Dissipation in Potatoes leaves in mg/Kg (Dry weight)

Day	Treated Potatoes leaves	Untreated Potatoes leaves
0	9.01±0.52	<LOD
2	4.05±0.24	<LOD
4	3.45±0.02	<LOD
7	1.72±0.06	<LOD
14	<LOD	<LOD
21	<LOD	<LOD
28	<LOD	<LOD

LOD: Limit of Detection = 0.05 mg/Kg

The trend of degradation obtained by plotting determined concentration of metalaxyl M versus time in days is shown in Figure 4.14. There was decrease in the concentration of metalaxyl M in potatoes leaves over time. 9.01±0.52 mg Kg^{-1} (day 0) was the average initial deposition of metalaxyl M, while 1.72±0.06 mg Kg^{-1} was the concentration measured on day 7. It was noted that after 2 days 44.95 % of metalaxyl M remained in the potatoes leaves while after 4 days, 38.29 % of the initial deposited pesticide remained in the potatoes. On day

7, only 19.09 % of the initial deposited metalaxyl M was detected, while no residue was detected after day 7. The results showed rapid dissipation of metalaxyl M in the potatoes leaves for the first 2 days followed by a slower second phase exhibiting a characteristic two-phase dissipation pattern.

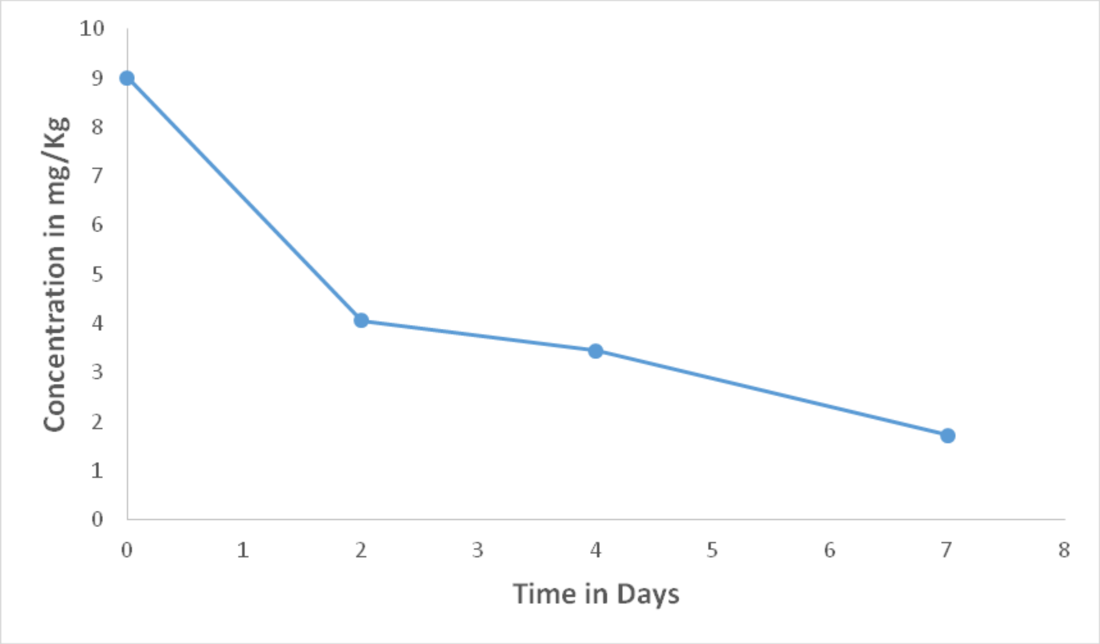


Figure 4. 14Trends of Metalaxyl M Dissipation in Potatoes leaves

The data was fitted into Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration (Kar *et al.*, 2013).

R α dC/dt = kKC/(1+KC)--(1)

Where, r = rate of reaction (mol/L.min),

C = equilibrium concentration of reagent (mol/ L),

t = time (min),

k = rate constant (1/min), and

K = Langmuir constant (L/mol).

When the initial concentration Co is <<<1, the denominator in equation 1 above can be assumed to be 1, and the equation becomes an apparent first-order equation when simplified to:

dC/dt=kKC--(2)

dC/C=kKdt---(3)

$dC/dt = -Kt$ ---------------------(4)

Integrating equation 3 and taking boundary conditions of C = Co at t=0, gives

Ln (Co/Ct)= kKt=k$_{abs}$------------------------------------ (5)

Or

Ct= Coe^{-kt} --- (6)

This is the first order rate equation, also written as:

$\ln C_t = \ln(C_0) - K_{obs} Xt$ ------------ -------(7)

Where; C_t= pesticide concentration at time, t

K_{obs}= first order rate constant

t= time in Days

C_o= the original Metalaxyl M concentration

Consider the half-life of the reaction where the remaining concentration of the pesticide is half the original amount; C_t= C_o/2 and substituting in equation 7 above gives:

$Ln(C_o/2C_o=-Kt_{1/2}$---------------------------------------(8)

$Ln\ 0.5=-Kt_{1/2}$ ---(9)

$-0.693/K=t_{1/2}$ ---(10)

The regression curve for the disappearance of metalaxyl M is shown in Figure 4.15 which was obtained by plotting negative logarithm of the concentration of residues against time. It had a correlation of $R^2 = 0.948$.

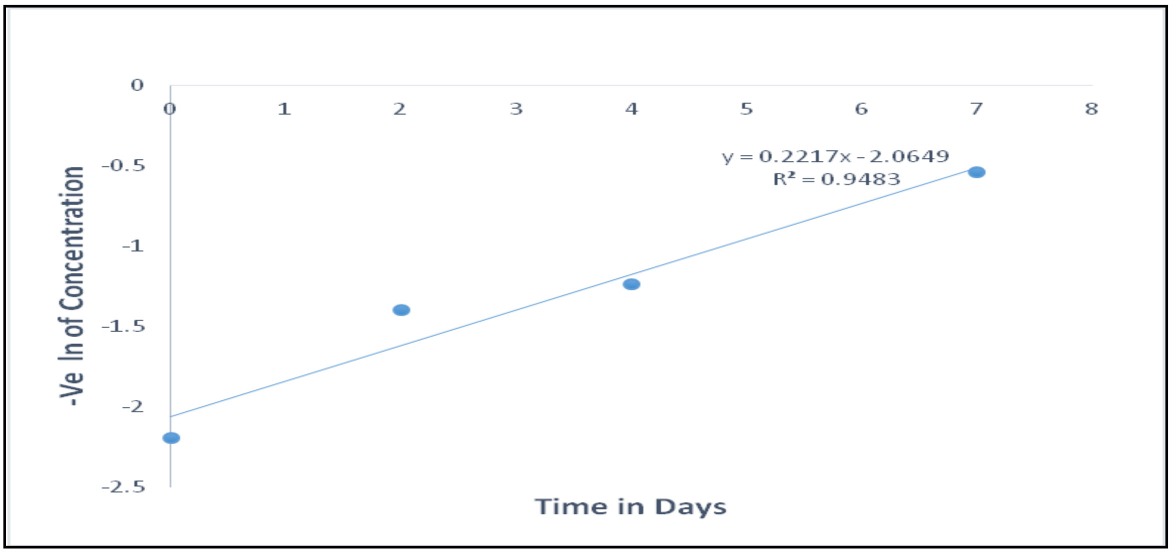


Figure 4. 15Regression curve for disappearance of Metalaxyl M from Potatoes

Equation 7 is in the form of y= mx + c. A plot of $\ln(C_t)$ verses time (t) gives a straight line, the slope of which upon linear regression equals the apparent first- order rate constant $K_{obs.}$ In this case, the K_{obs} are the observed degradation rate constant. The values obtained by this analysis were the same as that of the exponential regression analysis. Based on first order kinetics, a plot of negative ln concentration of residues versus time t (days) is shown in Figure 4.15 was made. The regression equation, $y = 0.2217x-2.0649$ gave a gradient of 0.2217 which was equivalent to the rate constant K_{obs}. The study revealed that degradation of metalaxyl M on potatoes leaves follows Langmuir-Hinshelwood kinetic equation and using equation 10, the half-life of metalaxyl M in potatoes leaves was 3.13 days. This value of half-life is significant in determining the efficacy of metalaxyl M againist fungal attack after application of the fungicide.

4.7 Dissipation of Metalaxyl M in Soil

The figure below shows the chromatogram of metalaxyl M residues in treated soil. Identification of the metalaxyl M was done using mass spectrometer model HP5771A and the NIST library 2008 that was also used to identify metalaxyl M in the standard.

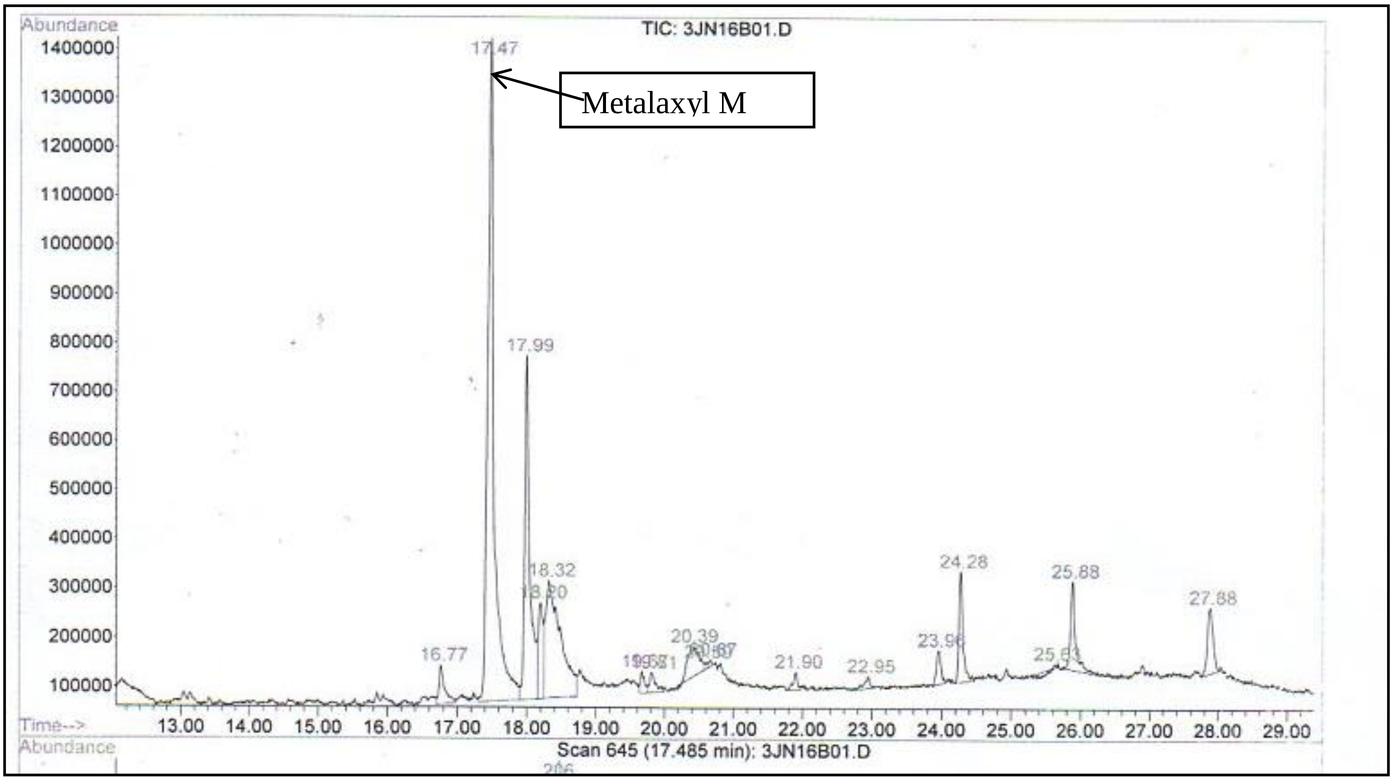


Figure 4.16 Chromatogram of Metalaxyl M Residues in treated Soil

This study was conducted to establish whether application of metalaxyl M on crops growing on treated soil result in elevated residue levels in soil. The results based on dry weight for the dissipation of metalaxyl M in soil are tabulated in Table 4.4.

Table 4.4 Results of Metalaxyl M Dissipation in Soil (mg/Kg, dw)

Day	Treated Soil	Untreated Soil
0	5.03±0.25	<LOD
2	2.52±0.01	<LOD
4	1.91±0.09	<LOD
7	1.23±0.02	<LOD
14	<LOD	<LOD
21	<LOD	<LOD
28	<LOD	<LOD

LOD: Limit of Detection = 0.05 mg/Kg

Figure 4.17 below shows dissipation of metalaxyl M residues in soil in the first few days. There was decrease in concentration of metalaxyl M in soil over time. 5.03±0.25 mg Kg^{-1} (day 0) was the average initial deposition of metalaxyl M and 1.23±0.02 on day 7 was the final residue deposition. The results showed that there was rapid dissipation of metalaxyl M in soil during the first 2 days and that the characteristic two-phase dissipation pattern showing the initial faster dissipation rate followed by slower rate starting after 2 days was seen.

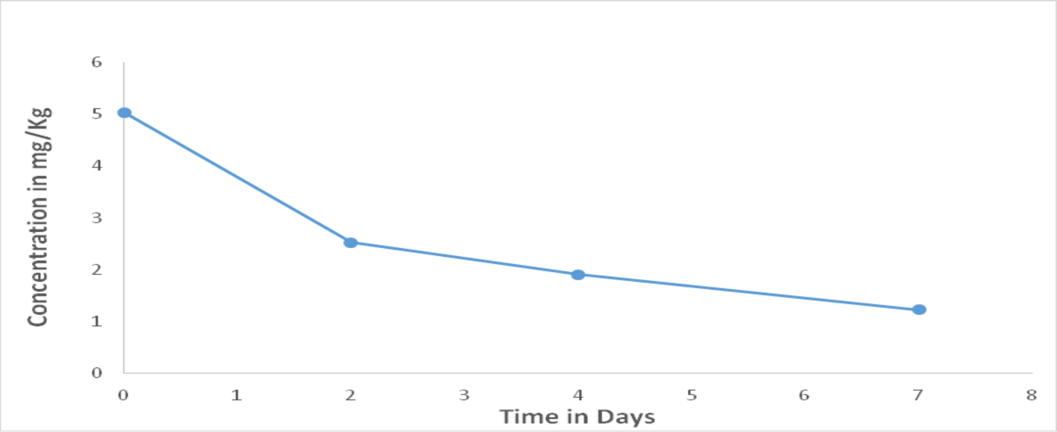


Figure 4. 17Trends of Metalaxyl M Dissipation in Soil

The data was fitted into Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration (Kar *et al.*, 2013). The regression curve for the disappearance of metalaxyl M in soil was obtained by plotting negative logarithm of the concentration of the residues against time. The value of R^2 was =0.946 (Figure 4.18).

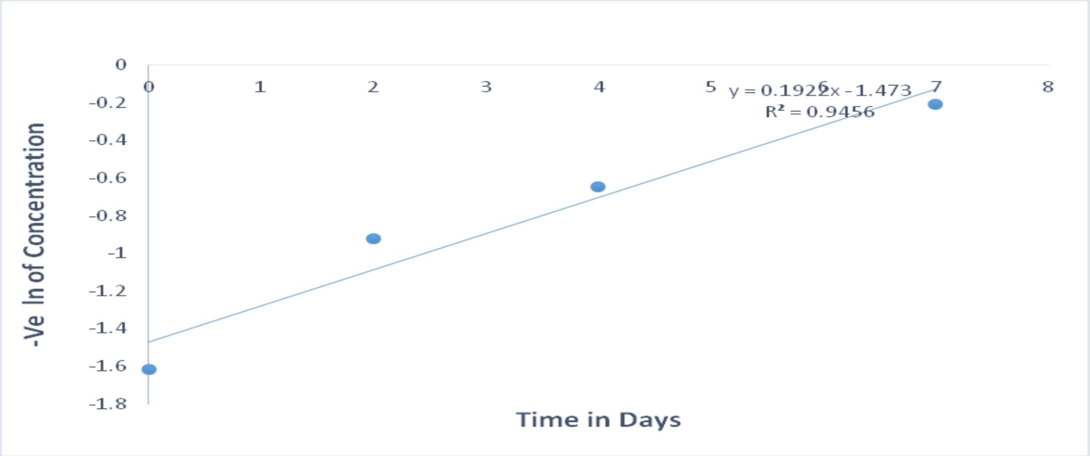


Figure 4. 18 Regression curve for disappearance of Metalaxyl M from Soil

Half-life of metalaxyl M in soil was calculated using equation 10 and data in Table 4.4 through regression analysis assuming the loss of metalaxyl M follows Langmuir-Hinshelwood kinetic model. K was determined (gradient=0.1922) and the half-life was found to be 3.61 days. The half-life of metalaxyl M was higher in soil than in potatoes indicating that metalaxyl M is more persistent in soil compared to the potatoes.

4.8 Dissipation of Thiamethoxam in Snow Peas leaves

The spray mixture applied to the snow peas contained thiamethoxam 250 g/Kg of actara 25WG. The control and the plants growing on untreated soil had non-detectable residues of thiamethoxam. Collected samples were analysed and the results tabulated in Table 4.5 below.

Table 4.5 Results of Thiamethoxam Dissipation in snow peas (mg/Kg, dw)

Day	Treated Snow Peas leaves	Untreated Snow Peas leaves
0	2.21±0.00	<LOD
2	1.86±0.00	<LOD
4	1.06±0.04	<LOD
7	0.68±0.01	<LOD
14	0.50±0.01	<LOD
21	0.47±0.03	<LOD
28	<LOD	<LOD

LOD: Limit of Detection = 0.01 mg/Kg

Figure 4.19 shows data for dissipation of thiamethoxam. The results revealed faster dissipation during the first four days, which was followed by a slower phase thereafter. The initial concentration of thiamethoxam was 2.21±0.00 mg Kg^{-1} (day 0) which reduced to 0.47±0.03 mg Kg^{-1} on day 21. A rapid dissipation of thiamethoxam in snow peas during the first 4 days and the characteristic two-phase dissipation pattern showing the initial faster dissipation rate followed by lower rate starting after 4 days were seen (Fig 4.19).

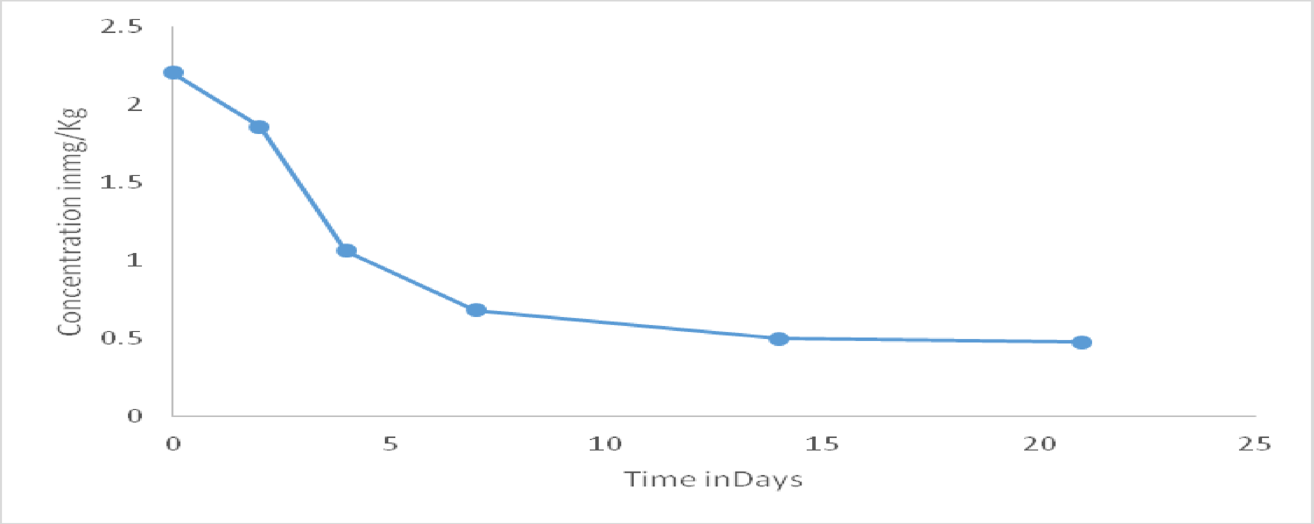


Figure 4.19 Trends of Thiamethoxam Dissipation in Snow Peas leaves

The data was fitted into Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration (Kar *et al.*, 2013). The regression curve for the dissipation of thiamethoxam in snow peas is shown in Figure 4.20 below which was obtained by plotting a graph of negative logarithm of the concentration of the residues against time. The value of R_2 was 0.8073 suggesting good correlation. (Figure 4.20).

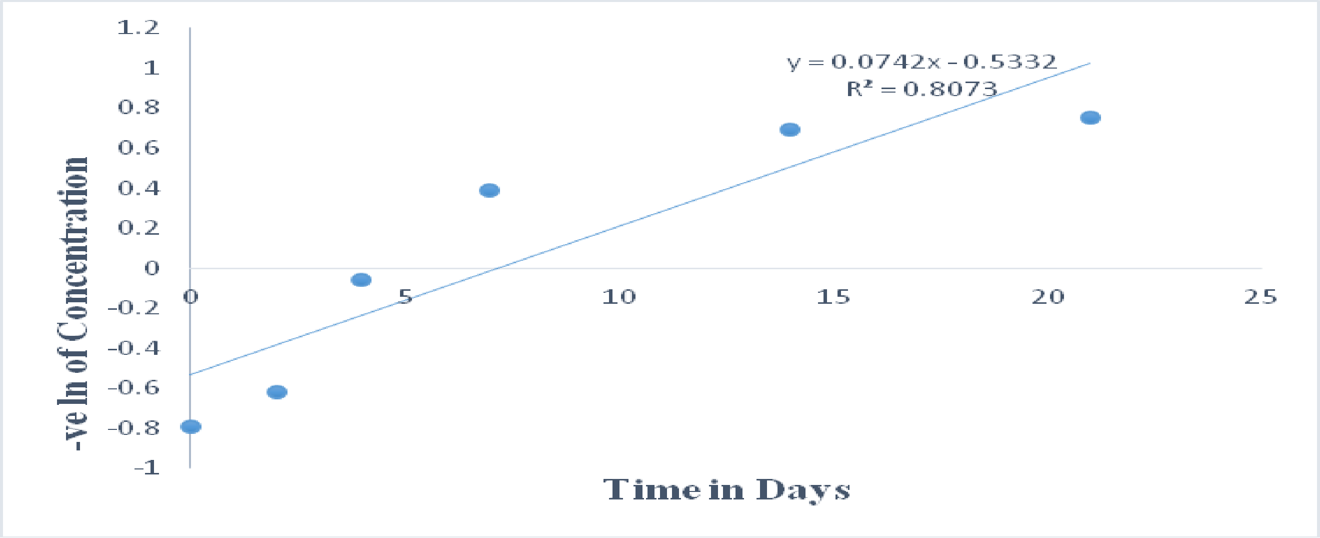


Figure 4. 20 Regression curve for disappearance of thiamethoxam from snow peas

Half-life of thiamethoxam in snow peas was calculated using equation 10 and data obtained in Table 4.5. The half-life ($t_{1/2}$) of thiamethoxam on snow peas was calculated from regression analysis assuming the loss of thiamethoxam followed Langmuir-Hinshelwood kinetic model, after k was determined (gradient=0.0742) and was found to be 9.34 days.

The concentration of thiamethoxam after the 21st day of pre-harvest interval (PHI) was calculated using the equation $C_t = C_o e^{-kt}$. Where, C_t is concentration of thiamethoxam at t =21 (PHI), C_o is concentration of thiamethoxam at t = 0 and the first order rate constant is represented by k. The concentration of thiamethoxam on the PHI day was found to be 0.465 mg/Kg for snow peas growing on untreated soil.

4.9 Dissipation of Thiamethoxam in Soil

The aim of this study was to establish whether application of thiamethoxam on soil at normal field conditions resulted in elevation or decrease of pesticide residue levels of thiamethoxam in soil. The results based on dry weight for the dissipation of thiamethoxam in soil are shown in Table 4.6.

Table 4.6 Results of Thiamethoxam Dissipation in Soil (mg/Kg, dw)

Day	Treated Soil	Untreated Soil
0	0.16±0.01	<LOD
2	0.13±0.01	<LOD
4	0.12±0.00	<LOD
7	0.08±0.00	<LOD
14	0.07±0.00	<LOD
21	0.05±0.00	<LOD
28	<LOD	<LOD

LOD: Limit of Detection = 0.01 mg/Kg

The data demonstrated that there was fast dissipation of thiamethoxam residues in the first week followed by a slower phase (Figure 4.21). 0.16±0.01 mg Kg^{-1} (day 0) was the average initial deposition of thiamethoxam and 0.05±0.00 mg/Kg on day 21 was the final residue deposition. The results showed that there was rapid dissipation of thiamethoxam in soil during the first 7 days and that the characteristic two-phase dissipation pattern showing the initial faster dissipation rate followed by lower rate starting after 7 days was seen.

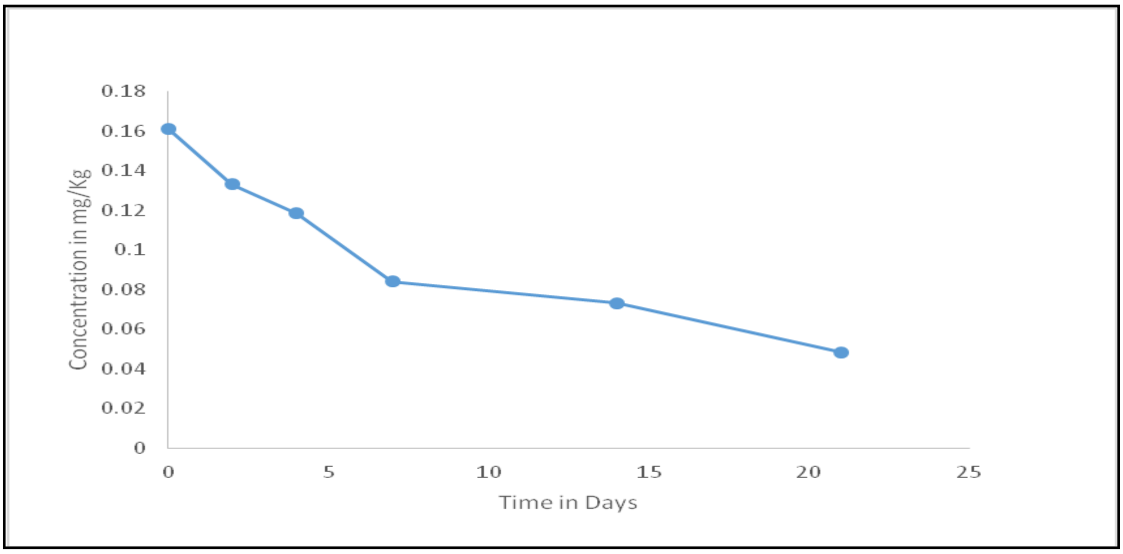


Figure 4. 21 Trend of thiamethoxam dissipation in soil

The regression curve for the disappearance of thiamethoxam from soil is shown below which was obtained by plotting a graph of negative logarithm of the concentration of the residues against time and it had correlation of R^2 =0.93 (Figure 4.22).

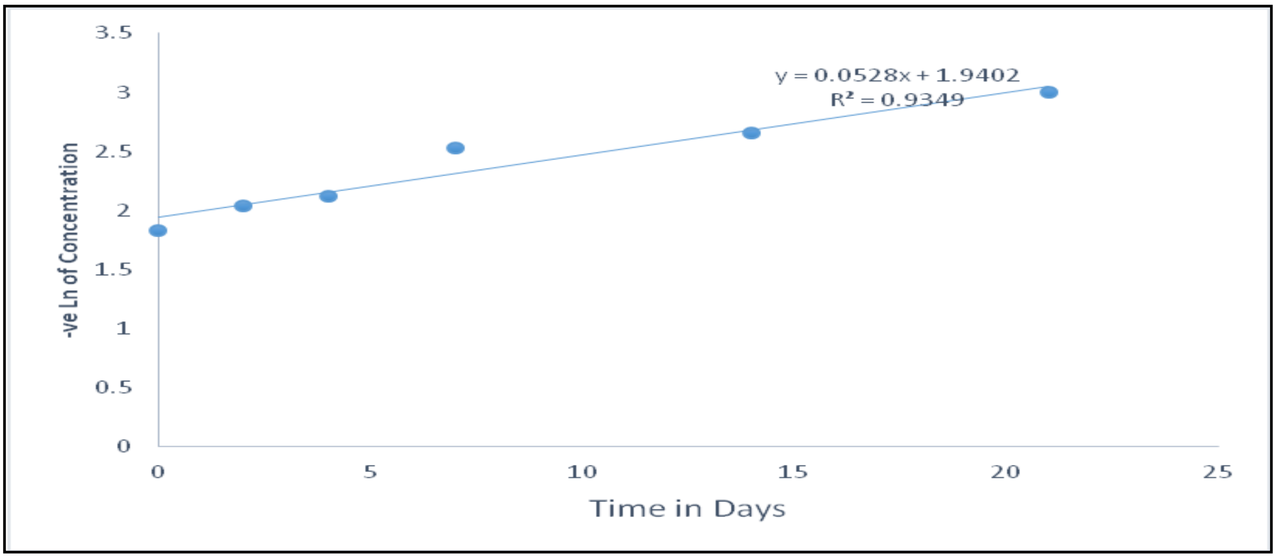


Figure 4. 22 Regression curve for disappearance of thiamethoxam from Soil

Half-life of thiamethoxam in soil was calculated using equation 10 and data obtained (Table 4.6), assuming the loss followed Langmuir-Hinshelwood kinetic model. The value of k was determined from the gradient to be 0.0528 and the half-life was found to be 13.13 days. The half-life of thiamethoxam was higher in soil than in snow peas suggesting that the pesticide persisted longer in soil than in snow peas.

4.10 Assessment of Metalaxyl M Residues in Potatoes

Samples of potatoes from three sampling markets namely Njabini, Kiburu, and Tulaga were analysed for Metalaxyl M residues. The results are presented in Table 4.7 below.

Table 4.7 Assessment of Metalaxyl M Residues in Potatoes mg/Kg (dry weight)

Season	Njabini	Kiburu	Tulaga
Wet (October-November)	<LOD	<LOD	<LOD
Dry (January-March)	<LOD	<LOD	<LOD

LOD = 0.05mg/Kg

The results show that the concentration of metalaxyl M was below the detection limits in all potato samples from all the three markets surveyed. This could be attributed to the fact that the GC-MS machine might be having high detection limit than the available pesticides residue in the food. This case is good since all the produce being sold in the three sites had below the European Union limit of 0.05 mg/Kg.

4.11 Assessment of Thiamethoxam Residues in Snow Peas

Samples of snow peas from three sampling markets (Njabini, Kiburu, and Tulaga) were analysed for thiamethoxam residues. The results are presented in Table 4.8 below.

Table 4.8 Assessment of Thiamethoxam Residues in Snow Peas mg/Kg (dry weight).

Season	Njabini	Kiburu	Tulaga
Wet (October-November)	0.44±0.01	0.41±0.06	<LOD
Dry (January-March)	0.49±0.00	0.46±0.03	0.43±0.01

The results show that thiamethoxam residues were detected in snow peas from two out of the three markets surveyed during the wet season and all the three markets during the dry season. Therefore, higher residues were observed during the dry season compared to the wet season (Figure 4.23). All detectable residues were below the MRLs (0.5 mg/Kg) according to the Eeropean Union standards.

Figure 4.23 Trends of thiamethoxam residues in snow peas at three markets

4.12 Discussion

Farmers in Kinangop sub-County control weeds, insects and pests using pesticides. The choice of a given pesticide by farmers depended on whether the pesticide enabled the farmer to get high yield as indicated by 48.4% of the respondents, cost effectiveness of the pesticide followed by 32.3% of the respondents noting it as an important factor. 4.8 % were those who used a particular pesticide because it was suggested by the agrochemical industries sales agent or agrochemical dealers. 9.7% of the respondents used pesticides because they were suggested to them by other sources, while 4.8% used pesticides because they thought pesticides were good for the environment. Out of the fifteen commonly used pesticides in Kinangop sub-County 7% were highly hazardous, 53% slightly hazardous and 40% moderately hazardous, based on the World Health Organization classification (WHO 2002).

Based on the responses to the questionnaires, there was low awareness among farmers about risks and safe handling of pesticides.42% of the farmers including those who had training on safe handling of pesticides did not use protective devices during pesticide application. The dose used and the spraying intervals seldom correlated with those recommended by the manufacturer. Most of the farmers did not follow the safety information and recommendations on pesticide dose because they did not read the instruction manuals, hence exposing both the farmers and the environment to risk.

Whereas 89 % of the farmers were aware of the effects of pesticides to the environment, 28 % of the farmers in Kinangop Sub-County were not aware of the ban or restriction imposed on some pesticides in Kenya, which are listed by the Pest Control Products Board (PCPB, 2008). Consequently, these pesticides may easily find their way into the region from other counties such as Nakuru and Kajiado where there have been reports of obsolete stock piles (NES, 2006).

In a previous study conducted by Abong’o *et al.* (2014) in the Nyando catchment, they documented agro-chemicals including pesticides used along the River Nyando drainage basin, recommended application rates, and the impacts on environment, human health and toxicity to birds and bees. In addition, the study found that organophosphate pesticides which were moderately hazardous were the most used pesticides in Nyando catchment area. However, some banned or restricted organochlorine pesticides were still used by some farmers. Therefore an active pest management programme was required to secure the harvest in the region against the major pests encountered in the area. The study, further observed that many farmers ignored safe use and handling of the pesticides, which could induce injuries and chronic illnesses (Abong’o *et al.*, 2014).

In my study of dissipation of metalaxyl M in soil, it was observed that the concentration decreased over time from 5.03±0.25 mg/kg (day 0) to 1.23±0.02 mg/Kg on day 7. The dissipation curve for the pesticide showed fast dissipation initially, followed by a slower rate. The half-life was found to be $t_{1/2}$ = 3.61 days. In the dissipation study of thiamethoxam in soil, the concentration decreased from 0.16±0.01 mg/kg (day 0) to 0.05±0.00 mg/Kg on day 21. The dissipation curve for the pesticide also showed a fast dissipation during the first week followed by slower second phase. The half-life was found to be $t_{1/2}$ =13.13 days. The processes responsible for the degradation of the two pesticides metalaxyl M and thiamethoxam could have been influenced by pesticide concentration, amount of rainfall, clay soil and organic matter. This is the reason the dissipation of metalaxyl M and thiamethoxam pesticides was initially fast due to high initial concentrations, and decreased over time with decreasing concentration. The high solubility of metalaxyl M in water suggested lower affinity for clay particles. The mobility of metalaxyl M in environment is variable and depends on rainfall, clay and organic matter contents of soils. Organic matter is the most important factor in metalaxyl M mobility in soil (Sharma and Awashi, 1997).

Soils with alkaline pH could favour hydrolysis of metalaxyl M, but this was not a factor in this study area because the soil was slightly acidic (Petrovic *et al.*, 1998). Sandy soils may not be able to retain metalaxyl M for an extended period of time, hence the fungicide could easily leach into groundwater ecosystems contaminating important water supplies. Therefore, precaution should be taken when metalaxyl is continuously applied to crops (Di and Ayimore, 1997).

The amount of residues at harvest could be influenced by factors such as the rate of application, time interval between last application and harvesting, and the type of crop. Other factors would include environmental factors such as temperature, precipitation and humidity and air movement (wind) which are known to influence the persistence of pesticides in plants and soil. The type of equipment, site and method of application together with the formulation type are key factors that determine the total quantity and form of pesticide reaching plant and soil. Consequently, these factors can also influence pesticide persistence on both the plants and soil (Edwards, 1975). Plant factors that influence persistence include plant species, structure of cuticle, nature of crop harvested, and metabolic activities like rate of uptake, growth, translocation storage and excretion (Edwards, 1975). In this study the observed pesticide residues levels during wet and dry season could be attributed to environmental factors since there was decrease in residue levels during wet season in snow peas when precipitation was high and temperature was low.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The results of this study suggest that:

An active pest management programme is necessary and important in Kinangop Sub- County to secure the harvest. This is because there are major pests' problems in this region.

Most of the pesticides used in farms in Kinangop Sub-County are organophosphates, carbamates and pyrethroids and some are highly hazardous. Most farmers ignore the safe use and handling of the pesticides, therefore training on pesticide handling is necessary for all the farmers.

Residue behavior of metalaxyl M in potatoes leaves and in soil, and thiamethoxam in snow peas and soil in the open field showed that both metalaxyl M and thiamethoxam were more persistent in soil than in potatoes and snow peas, respectively. The half-life of metalaxyl M was 3.13 days and 3.61 days in potatoes and soil, respectively, while that of thiamethoxam was 9.34 days and 13.13 days in snow peas and soil, respectively.

Metalaxyl M residues were below the limit of detection after day 7, suggesting that after day 7 the pesticide was not effective to control the early and late blight in potatoes leaves. All the potato samples collected from the three open air markets (Njabini, Kiburu and Tulaga) had metalaxyl M residues below detection limit.

The concentration of thiamethoxam in samples of snow peas taken from the three markets was higher during the dry season compared to the wet season. The levels in the wet season were 0.44 mg/Kg, 0.41 mg/Kg and <LOD in Njabini, Kiburu and Tulaga, respectively, while

during the dry season the levels were 0.49 mg/Kg, 0.46 mg/Kg and 0.43 mg/Kg in Njabini, Kiburu and Tulaga, respectively. Thiamethoxam residue on the pre-harvest day was 0.465 mg/Kg which was lower than both the EU and Codex MRLs. This showed that when the pesticide is applied in the right way it would not result into elevated levels above required limits during the time of harvesting.

It was found that after 7 days metalaxyl M was not detected in the potatoes leaves, therefore farmers are advised to spray the pesticide once every week for effective control of early and late bright. There were no Metalaxyl M residues detected in all the samples of potatoes analysed indicating that the potatoes are safe for consumption if they are sprayed at recommended dosage and harvested at normal harvesting interval of 14 days.

5.2 Recommendations

The recommendations of this study are that:

Farmers should be advised to carefully follow the manufacturers' instructions or specifications before and after applying the metalaxyl M, thiamethoxam and other pesticides on their vegetables

All samples from the three sampling sites (Njabini, Kiburu and Tulaga) had concentration of thiamethoxam residues lower than the EU MRLs of 0.5mg/Kg, therefore the snow peas from the area could be safe for consumption and export.

There should be a national pesticide residues monitoring and surveillance on produce sold in Kenyan markets and those destined for export to protect consumers, assessment of the implementation of GAP and detection of unauthorized or misuse of pesticides.

Farmers should be encouraged to avoid using pesticides when it is not necessary or to use alternative methods of pest control, for example the biological methods of pesticides control instead of thiamethoxam.

More sampling times is recommended for dissipation studies on multiple application of metalaxyl M, with two day sampling programme to monitor pesticide degradation.

Further research is recommended on the existence of metalaxyl M resistance strain of fungus in the study area.

From the results of this study and other studies conducted in Kenya, it would be important to monitor and ascertain the effectiveness of pesticides applied on crops, and pesticides residue levels in soil and horticultures crops to improve the quality of pesticides in the market.

REFERENCES

Abong'o D. A, Wandiga S. O, Jumba I. O., Madadi V. O. and Kyline Henrik., (2014) Impacts of pesticides on Human health and environment in the River Nyando catchment, Kenya. *International Journal of Humanities, Arts, Medicine and Sciences* **2**(3):1-14.

Atsukoa, A. and Toshio, O., (2006) pesticide residue reduction in selected vegetables using rice-bran. *Journal of Health science*, 52:320-323

Bempah, C. K. and A K. Donkor, (2010) Pesticide residues in fruits at the market levels in Accra Metropolis, Ghana, Preliminary study. *Environ. Monit. Assess*. 175:551-561.

Bempah, C. K., A.K Donkor, P.O. Yeboah, B. Dubey and P. Osei-Fosu, (2011). A preliminary assessment of consumer's exposure to organochlorine pesticides in fruits and vegetables and the potential health risk in Accra metropolis, Ghana. *Food chem,* 128:1058-1065.

Bernaerts, S. and L. pussemier, (1997) *Estimation of pesticide emission to surface and groundwater in Belgium using the SEPTWA95 model*. Medical Faculty Landbouw, University Gent 62: 157-170

Blasco C. Fernandez M. Pena A. Lino C. Silveira M. Font G. and Pico Y., (2003) Assessment of the pesticide residues in honey samples from Portugal and Spain. *Journal of agriculture and food chemistry*.51 (27):8132-8.

Botisi, H.V., Garbis, S.D., Economou, A. and Tsip, D.F., (2011) current mass spectrometry strategies for the analysis of pesticides and their metabolites in food and water matrices. *Mass spectrum Rer,* 30: 907-939.

Briggs, S. A., (1992) *Basic guide to pesticide their characteristics and hazards,* Rachel Carson Council, Washington D.C, USA.

Bursic, V., Lazic's, Vukovic, S., Sunjka, D. and Pucarevic, M., (2009) Disappearance of azoxzstrobin in cucumber. *Contemporary Agriculture*. 3(4): 81-86.

Chapman, R.A., C.M. Tu, C.A. Harris and C. Coli, (1981) Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil. *Bull. Environ. Contam. Toxicol.,* 26: 513-519.

Chen, C. N., German, T. Y. and Lourdes, D. M., (2006) *Monitoring of Pesticides Residues in Fresh Fruits and Vegetables in the Philippians, 2000-2005.* 11th IUPAC international congress of pesticides chemistry. Book of abstracts (2) for poster session. Port Island, Kobe, Japan pp209.

Chen, L, Shang, G. L., Wu, Y, Xu L, and Fu, F., (2012) study on the residue and degradation of fluorine-containing pesticides in Oolong tea by using gas chromatography-mass spectrometry .*Food control* 25:433-440.

Claeys, W., S. Jean-Francois, C. Bragard, G. Maghuin-Rogister, pLuc and B. schiffers, (2011) Exposure of several Belgian Consumer groups to pesticide residues through fresh fruit and vegetable consumption. *Food cont.*, 22:508-516.

Collins, D. A., (2006) A review of alternatives to organophosphorus compounds for the control of storage mites. *Journal of Stored Products Research*; 42: 395-426.

Department of Agriculture and Food Pesticide Control Services (DAFPCS), (2006) Report Pesticide residues in food 2004. Back Weston Camp us, young's cross, celbridg e, co Kildare, Ireland.

Dey, T, K. and M.S. Ali, (1994) Pathological research on tuber crops in Bangladesh. In: proc of workshop on transf. of Tech of CDP crops under Res. Ext linkage program, held on oct.22-27 BARI GAZIPUL Bangladesh. 159-165.

Di, H.J. and Aylmore, L.A.G., (1997) Modeling the probabilities of groundwater contamination by pesticides. *Soil Science Society of America Journal*, 61: 17-23.

Dorough, H. W. and Casida, J. E., (1964) Nature of certain carbamate metabolites of the insecticides, *J. Assoc. Off. Agric. Chem., 48(5): 927-937*.

Duke, J.A., (1981) Handbook of Legumes of World Economic Importance. Plenum Press, New York.pp. 199-265.

Edwards, C.A., (1975) Factors that Affect the Persistence of Pesticides in Plants and Soils. Rothamsted Experimental Station, Harpenden Herts., UK.

EFSA, (2010) 2008 Annual report 0n pesticide residues according to article 32 of regulation (EG) No396/2005. EFSA journal 8(6): 1646.

EFSA, (2011) Review of the maximum residue levels (MRLs) for metalaxyl-M according to Article 12 of regulation (EC) No 396/2005. EFSA Journal; 9(12):2494. [74 pp.]doi:10.2903/j.efsa.2011.2494: www.efsa.europa.eu/efsajounal

EFSA, (2015) (European Food Safety Authority). Reasoned opinion on combined review of the existing maximum residue levels (MRLs) for the active substances metalaxyl and metalaxyl-M. *EFSA Journal;* 13(4):4076, 56 pp. doi:10.2903/j.efsa.4076

European Union, (2005) commission regulation (EC) No 396/2005 of the European parliament and the council of 23 February 2005 on maximum residue in or on food and feed of plant and animal origin and amending council directive 91/414/EECText with EEA relevance. L70; 1-16.

Export Processing Zones Authority (EPZA), (2005) Report on Horticulture Industry in Kenya, pp 9. Nairobi, Kenya.

FAO, (1982) Data and recommendations of the joint meeting of the FAO panel of experts on pesticide residues in food and the Environment and the WHO Expert Group on pesticide residues Rome, 23 November- 2 December 1982.

FAO, (1998) Report of the joint meeting of FAO panel of experts on pesticide residues in food and environment and the WHO expert group on pesticide residues. FAO plant production and protection paper 133. FAO, Rome.

Fernandez-Alba, A.R. and Garcia-Reyes, J.F., (2008) Large scale multi residue methods for pesticide and their products in food by advanced LC-MS. *Trac-Trend. Anal. Chem*., 27 (11): 973-990.

Filho, A.M., Dos Santos, F.N, and de Paula P.P.A., (2010) Development validation and application of a methodology based on solid- phase micro extraction followed by gas chromatography coupled to mass spectrometry (SPME/GC-MS0 for the determination of pesticide residue in mangoes. *Talanta* 81:346-354.

Getenga, Z. M., Jondiko, J. I. O., Wandiga, S. O. and Beck, E., (2000) The Dissipation behaviour of Malathion and dimethoate from the garden pea plant (Pisum sativa). *Bull. Environ. Contam. Toxicol*. 64, 339 - 367.

Gilden, R. C; Huffling, K. and Sattler B (2010). Pesticides and Health Risks. *JOGNN*, 39, 103-110.

GOK, (2005) Provincial Horticulture Annual Report. MOA (Ministry OF Agriculture). Nairobi Kenya.

GOK, (2010) Provincial Horticulture Annual Report. MOA (Ministry OF Agriculture). Nairobi Kenya.

Guilhermino, L., Soares, A., Tinoco-Ojanguren, R. and Osten, J., (2004) Effect of pesticide exposure on acetyl cholinesterase activity in subsistence farmers from Campeche, Mexico. *Archives of Environmental Health*; 3: 1-30.

Hanafi, A., Garad, V.L., Caboni, P., Sarais, G. and Cabras, P., (2010) minor crops for export: a case study of boscolid, pyraclostrobin, lufenuron and Lambda-cyhalothin residue levels on green beans and spring onions in *Egypt J environ sci Health part* B 45:493-500.

Harris, J., (2000). *Chemical pesticide markets*, Health Risks and Residual. CABI, Wallingford.

Hassall, A. K. (1990). The Biochemistry and Uses of Pesticides – Structures Metabolism, Mode of Action and Use in Crop Protection. Macmillan press Ltd, London.

HCDA, (2014). Horticultural Crops Development Authority, United States Agency for International Development (USAID) and Ministry of Agriculture (MOA). Horticulture Validated Report 2013.

Howard, p. H., R .S. Boething, W. F. Jarvis, W, M. Meylan and E.M. Michalenko. (1991). Handbook of Environmental Degradation Rates. Lewis publishers, Ann Arbor, Michigan, USA Impact of pesticides. *New York Food and life science Bulletin,* 139: 1-8.

Idowu, G.A., Aiyesanmi, A.F., and Owolabi, B.J., (2013). Organochlorine pesticide residue levels in river water and sediments from cocoa-producing areas of ondo estate central senatorial district, Nigeria.

Jeyanthi, H. and Kombairaju, S., (2005) Pesticide use in vegetable crops: Frequency, Intensity and determinant factors. Agricultural Economics Research Review 18:209-221.

Jobling S., Reynolds T., White R., Parker M. and Sumpter J., (1995) Chemicals found to mimic human estrogens. Environmental Health Perspectives, 103: 582-587.

Juraske, R., C. mutel, F. stoessel and S. Hell weg, (2009) Life Cycle human toxicity assessment of pesticides: Comparing fruit and vegetable diets in Switzerland and the United States. Chemosphere, 77:939-945.

Kar, R., Oindrila, G., Kunal, M., and Sangita, B., (2013) Performance Study on Photocatalysis of Phenol Solution in a UV Irradiated Reactor. J. Chem. Eng. Process Technol, 4:1.

Kegley, S. and Hills, B., (2007) PAN Pesticides Database, pesticide Action Network, North America (San Francisco, CA).

Kenya Economics Survey Report, (2012) Kenya National Bureau of Statistics

KLR, (2010). Kenya Law Reports, Laws of Kenya, The Public Health act, Cap 242; The Plant Protection Act, Cap 324; The Agriculture Act, Cap 318, The Agricultural Produce (Export) Act, Cap 319, The pest control products Act, Cap 346 and The food, Drugs and Chemical Substances Act, Cap 254 Revised edition 1992 (1980) http://www.kenyalaw.org/kenyalaw/klr_home/ Accessed on 16th May 2016.

KMD, (2009). Kenya Meteorological Department, Review of Rainfall During the "Long Rains" (March to May) 2009 Season and the Outlook for the June- July- August 2009 period. Ref.No.Met/7/23.

Kenya Plant Inspectorate services (KEPHIS), (2006) Annual Report, July 2005-June 2006; Nairobi, Kenya.

Kovach, J., C. Petzoldt, J. Degni and J. Tette, (1992) A method to measure the environmental pollution.

Lartiges S. and B., Garrigues, (1995) Degradation kinetics of organophosphorus and organonitrogen pesticides in different water under various environmental conditions. Environmental science and Technology, 29:1246-1254.

Lemaire, A., Burat-Herbaut M. and Marcolin G., (2004) Endocrine Screening in men, clinical significance and cost effective strategy, urol.

Leong, K.H., Tan, L.B. and Mustaga A.M., (2007) *Contamination levels of selected organochlorine and organophosphate pesticide in Selengor River, Malaysia..*

Lewis, K., C. D. Brown., A. Hart and J. Tzilivakis, (2003) P-EMA (III). Overview and application of a software system designed to assess the environmental risk of agricultural pesticide. *Agromie* 23: 85-96.

MacBean, C., (2010) Thiamethoxam e-pesticide Manual, 15th ed. Version 5.0.1.Survey UK, British Crop protection council. (153719-23-4).

MacLachlan, D.J. and Hamilton, D., (2010) Estimation method for maximum residue limit for pesticides. *Reg Toxicol pharmacol* 58: 208-218.

Mansour S.A., (2004) Pesticide exposure – *Egyptian scene. Toxicology*, 198: 91-115.

Matthews, G.A., (2006) *Pesticides*: Health, safety and the Environment Blackwell publishing,

MOA, (1980). Ministry of Agriculture, National Agriculture Laboratory (MoA, NAL). Physical and Chemical Methods of Soil Analysis. Nairobi, Kenya

MOA, (2005). Ministry of Agriculture, Provincial Annual Report. Nairobi, Kenya

MTTI, (2007). Ministry of Tourism, Trade and Industry, Government of Kenya, Report on Republic of Kenya Fertilizer and Pesticides Sub-Sector Profile and Opportunities for Private Investment Page 25. Nairobi, Kenya.

Mullen, J.D., (1995) *Estimating environmental and human benefits of reducing pesticide use, through integrated pest management programmes*. MSC Thesis. Virginia polytechnic institute and state university Blacksburg, Virginia, USA.

Muraguri, J., Mutwiri, K., Agawo, D., Arjan, M., Mbogo S., Juma, V. and Muli, J., (2012) Kenya Agricultural Research Institute stunning success in potato breeding. *Hortfresh Journal.*

Musila, A., (2011) *Fate of Chlorothalonil on snowpeas and assessment of its residues in French Beans and Passion Fruits in Nairobi*. M.sc. Thesis, Department of Chemistry, University of Nairobi, Nairobi, Kenya.

Mwangi, T., (2013) Threat to market access for Kenya’s beans and snow peas to European Union, *Hortfresh Journal*

NEAP, (1994) Report, Kenya National Environmental Action Plan, Ministry of Environment and Natural Resources, Nairobi, Kenya.

NES, (2006) National Implication Plan for Stockholm convention on Persistent Organic Pollutants. MENR, Nairobi, Kenya.

Njagi, J., (2005) *Analysis of Deltamethrin and lambdacyhalothin pesticides in selected vegatables from Urban and Rural areas in Kenya* M,sc. Thesis, Department of Chemistry, Kenyatta University. Kenya.

OECD, (2010) *OECD Survey on pesticide maximum residue limit (MRL) policies: survey results.* OECD series on pesticides, No. 51.

Okero, J. J. and Swinton, S. M., (2010) From circle of poison to circle of virtue: Pesticides, export standard and Kenyan's green bean farmers. *Journal of Agriculture Economics,* 61, 209-224.

Okello, J., Ofwona-Adera E., Mbatia, O. and Okello, R., (2014) Using ICT to integrate Smallholder Farmers into Agricultural Value Chain: The Case of DrumNet Project in Kenya. *International Journal of ICT Research and Development in Africa,* (1): 23-37.

Omirou, M., Vryzasz, P and E., Economou, (2009) Dissipation rates of iprodione and thiacloprid during tomato production in greenhouse. *Food Chem* 116:499-504. Oxford. UK.

Oudejans, J. H., (1991) *Agro-Pesticides Properties and Functions in Integrated Crop Protection.* Agricultural Requisites Scheme for Asia and the Pacific, Bangkok, Thailand.

PCPB, (2004) *Annual Report, July 2003/June 2004,* Pest Control Products Board, Nairobi, Kenya.

PCPB, (2005). (Pest Control Products Board), *Annual report*. Nairobi. Kenya.

PCPB, (2008). *Annual Report, July 2007/June 2008*, Pest Control Products Board, Nairobi, Kenya.

Pesticide Action Network, (2001) The hazards of pesticides use for man and the ecosystem, pesticide in tropical Agriculture. pg. 23-61

Petrovic, A. M., Barret, W.C., Larsson-Kovach, I.M., Reid, C.M. and Lisk, D.J., (1998) Downward migration of metalaxyl fungicide in creeping bentgrass and lysimeters as affected by organic waste, peat and zeolite amendments. *Chemosphere***,** 37: 249-256.

Podolska, M. and Napierska, D., (2006) Acetyl cholinesterase activity in hosts (herring Clupea harengus) and parasites (Anisakis simplex larvae) from the southern Baltic. ICES (International Council for the Exploration of the Sea). *Journal of Marine Science;* 63(1): 161-168.

Repeto, R. and S. S. Baliga, (1996) *Pesticides and immune system.* The public health risks. World Institute, Washington, DC, USA.

Reus, J., P. Leendertse, c. Bockstaller, I. Fomsgaard, V. Gutsch, K. Lewis, C. Nilson, L. Pussemier, M.Trevisan, H. Van der werf, F. Alfarroba, s. Blumel, J. Isart, D .McGrath and T. Seppala. (1999) *Comparing environmental risk indicator for pesticides, Results of the European CAPER Project*. Center for agriculture and environment, Utrecht, the Netherlands. Report CLM 426

Runno, E., and Koppel, M. (2005) Phytophthora infectans, 220, 168-170

Schwinn, F.J. and Staub, T., (1987) Phenylamide and other fungicides against Oomycetes.in lyr. H. (ed). Modern selective fungicides. Jena: VEB Gustar Fischer, pp 259-73.

Sharma, D. and Awasthi, M.D., (1997). Adsorption and movement of metalaxyl in soils under unsaturated flow conditions. *Plant and Soil,* 195:293-298.

Slinkard, A.E., Bascur G. and Hernandez- Bravo G., (1994) Biotic and Abiotic Stresses of Cool Season Food Legumes in Western Hemisphere. Pp. 195-203. In: F.J.Muehlbauer and W. J. Kaiser (eds), Expanding the Production and Use of Cool Season Food Legumes. Kluwer Academic Publishers. Dordrecht, Netherlands.

Souza, M. V. and Caldas, E.D., (2006) *Residues of Dithiocarbamates and organophosphorus Pesticides in Food for a Vegetarian Meal and Non- Vegetarian Diet in Brasilia, Brazil. 11th IUPAC International Congress of Pesticide Chemistry. Book of Abstract (2) for Poster Session*. Port Island, Kobe, Japan pp 209.

Turgut C., (2007) The impact of pesticides toward parrot feather when applied at the predicted environmental concentration. *Chemosphere,* 66 (3): 469 – 473.

USEPA (2009) *Health and environmental effects profile for carbaryl.* Cincinnati, USA.

Van der werf, H. M. G. and C. Zimmer, (1998) An indicator of pesticide environment impact based on a fuzzy export system. *Chemosphere,* 36: 2225-2249.

Vesna, K., Bistra, A., Evgenijia, K. and Biljana, B., (2014) Determination of pesticide Residue in plant-based foods from the Republic of Macedonia. *Journal of Food and Nutrition science*. 2 (4):124-129.

Vesna, Weber C.I, G.H. Muresah, B. Georgescu, (2001) Organochlorine pesticide residues analysis from cow milk.

Vidal, M. J., Arrebola, F.J. and Mateu-Sanchez, M., (2002) Application of gas chromatography- tandem mass spectrometry to the analysis of pesticide in fruits and vegetables, *Journal of chromatography* A, 959, 203-213-2002.

Wandiga, S.O., Lalah, J.O. and Kaigwara P.N., (2003) Pesticides in Kenya, In: Pesticide Residues in Coastal Tropical Ecosystems; Distribution, Fate, and Effects. Taylor M.D, Klaine S. J Carvalho F. P, damia B. and Everaarts 4, pp 49-80.

Wasswa, J., (2008) *Characterization of Selected Pesticides Residues in Sediments of the Ugandan side of Lake Victoria*. PhD Thesis, Makerere University, Uganda.

WHO, (2002) Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1998-1999.

WHO, (2006) Pesticide data sheets. http://www.inchem.org/pages/pds.html.

WHO, (2008) pesticide, Retrieved July 20, 2015 from http://www.who.int/ceh/capacity/pesticide.

YAEI, (2012). Youth Agro-Environment Initiative Small scale potatoes growing business in Kenya, 2012. Available online at http://yagrein.blogspot.com/2012/05/small-scale-potatoesgrowing-business.html [Accessed: November 2015]

APPENDICES

Appendix I

TABLE A1 Test soil Fertility evaluation Parameters and Physicochemical properties

Parameters	Test Soil	Critical level	Comment in relation to potato and snow peas growing
Ph	6.50	5.5	Slightly Acidic
Total Nitrogen %	0.52	0.2	Adequate
Organic carbon %	3.62	0.5	Adequate
Phosphorous (ppm)	27.5	30ppm (Mehlich 1)	Low
Potassium (me %)	1.23	0.2	Adequate
Calcium (me %)	4.7	2.0	Adequate
Magnesium me %	2.22	1.0	Adequate
Manganese me%	0.35	0.11	Adequate
Copper (ppm)	2.13	1.0	Adequate
Iron (ppm)	71.5	10.0	Adequate
Zinc (ppm)	4.38	5.0	Low
Sodium me %	0.10	2	Adequate
Clay %	77	40	Clay soil, texture grade C
Silt %	15.9	-	
Sand %	7.1	-	
Bulk Density	0.72	-	
PF 0	84.48	-	Saturation point
PF 2	62.17	-	Field capacity
PF 2.3	42.67	-	Field capacity
PF 4.2	20.45	-	Permanent wilting point

APPENDIX II

QUESTIONAIRE ON THE USE OF PESTICIDES IN KINANGOP, NYANDARUA COUNTY

Introduction

This questionnaire seeks to gather information on knowledge concerning the types and use of pesticides in Kinangop, Nyandarua County. Please note that this is not a test and there are no wrong and right answers. If you have no answer for a particular question, be free to write" I don't know''. Your time and honest opinion will be highly appreciated

SECTION 1; General information (Kindly fill where applicable)

Questionnaire No…………………………….Date……………………………………………

Address……………………………………………………………………………….

Gender, Male……………………….Female……………………………………….

Age…………………………………......Occupation…………………………………

For how long have you lived in this area…………………………………..

The highest level of education reached, please tick.

Primary ☐

Secondary ☐

Post-secondary ☐

Section 2: Pesticides used in Engineer, Nyandarua County.

Do you have any information on the chemicals used in this area for splaying crops? Please tick

Yes ☐

No ☐

Please if yes, list some of the chemicals ………………………………………………………………………………………… ………………………………………………………………………………………… ………………………………………………………………………………………… ………………………………………………………………………………………… ……………………

Are there any guidelines given to you before buying any pesticide chemicals? ....who give the guidelines ……………………………………………

Do you read and understand the instructions and procedures before using pesticides?

Which factors do you consider when choosing a given pesticide?.. ....

Have you ever had any training on pesticide management and safety? ………………………………………………………………………..

In which institution were you trained? ....

How long did the training take? ....

What role did the Government play in your training……………………

Do you use any form of protection when handling pesticide chemicals? .... If yes which one? ....

Do you wash your hands after using pesticides? Please tick.

Yes ( ) No ( )

Do you change clothes after using pesticides? Please tick.

Yes ( ) No ( )

Do you shower after splaying chemicals? Please tick

Yes ( ) No ( )

Where do you dispose used pesticide containers? ....

Where do you dispose expired products? ……………………………………..………………..

Where do you dispose solutions not used? ....

Do you have information on the pesticide related pollution in this area? .... If yes give some details ………………………………………………………………………………………………… ………………………………………………………………………………………………… ………………………………………………………………………..

Do you suffer from any illness? Yes ( ), No ( ).

If yes specify the kind of illness………………………………………………………………………………………… …………………………………………………………………

Do you know any national body that regulate the use of pesticides in Kenya? ....If yes write its name ……………………………………………………… …

Do you know of any banned or restricted pesticides in Kenya?

After how long do you harvest the crop after pesticide application?

…………………………………………………………………..

Overall how do you rate the effectiveness of the pesticides?

i) Poor ( ) ii) fair ( )

iii) Good ( ) iv) very good ( )

What are the challenges you face while using pesticides

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

…………………

Section 3: chemical Dealers

Have you ever been trained on how pesticides can be used in safe manner? ....

List chemicals that are commonly purchased by farmers

……………………………………………………………………………

…………………………………………………………………………………

…………………………………………………………………………………

…………………………………………………………………………………

…………………………………………………………………………………

Do you offer any after sale services or technical advice to your clients? ....

If yes, indicate the kind of service or advice you offer (tick the one applicable).

Dosage ( )

Safety ( )

Disposal of used containers ( )

Others specify ( )

Do you know any rule and regulations for pesticide handling in Kenya? ....

How are these rules enforced by the Government?...

....

When do you have farmers seminars? ....Who comes to facilitate? ....

Section 4: extension officers

Do you have regular training sessions for use of pesticides for the farmers?

Yes (   )

No   (   )

How often are the training sessions?

Monthly (   )

Annually (   )

Others (   ) specify………………………

What are the common challenges of the farmers in the use of pesticides?

Thank you for your responses.

APPENDIX III

Table A2: Questionnaires Analysis.

<table>
<tr><td colspan="7">Percentage of Questionnaires returned.</td></tr>
<tr><td rowspan="3"></td><td colspan="6">SAMPLING AREA</td></tr>
<tr><td colspan="2">KIBURU</td><td colspan="2">NJABINI</td><td colspan="2">TULAGA</td></tr>
<tr><td>ISSUED</td><td>RETURNED</td><td>ISSUED</td><td>RETURNED</td><td>ISSUED</td><td>RETURNED</td></tr>
<tr><td>FARMERS</td><td>25</td><td>20</td><td>30</td><td>24</td><td>25</td><td>18</td></tr>
<tr><td>CHEMICAL DEALERS</td><td>3</td><td>1</td><td>8</td><td>5</td><td>4</td><td>2</td></tr>
<tr><td>EXTENSION OFFICERS</td><td>1</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td></tr>
<tr><td>TOTAL</td><td>29</td><td>22</td><td>40</td><td>31</td><td>31</td><td>21</td></tr>
<tr><td>PERCENTAGE (%)</td><td>100</td><td>75.862069</td><td>100</td><td>77.5</td><td>100</td><td>67.741936</td></tr>
<tr><td colspan="7">Respondent’s gender</td></tr>
<tr><td>SAMPLING AREA</td><td colspan="2">KIBURU</td><td colspan="2">NJABINI</td><td colspan="2">TULAGA</td></tr>
<tr><td rowspan="2">GENDER</td><td>MALE</td><td>FEMALE</td><td>MALE</td><td>FEMALE</td><td>MALE</td><td>FEMALE</td></tr>
<tr><td>18</td><td>4</td><td>20</td><td>11</td><td>10</td><td>11</td></tr>
<tr><td colspan="7">Respondent’s age</td></tr>
<tr><td>AGE</td><td>18-24</td><td>25-35</td><td colspan="2">36-45</td><td colspan="2">45 & above</td></tr>
<tr><td>FARMERS</td><td>10</td><td>15</td><td colspan="2">28</td><td colspan="2">9</td></tr>
<tr><td>CHEMICAL DEALERS</td><td>_</td><td>2</td><td colspan="2">6</td><td colspan="2">_</td></tr>
<tr><td>EXTENSION OFFICER</td><td>_</td><td>_</td><td colspan="2">3</td><td colspan="2">1</td></tr>
<tr><td colspan="7">Education level of the respondent’s</td></tr>
<tr><td></td><td colspan="4">NO OF RESPONDENTS</td><td colspan="2">PERCENTAGE (%)</td></tr>
</table>

PRIMARY	15	Kiburu	Njabini	Tulaga	20.27
		5	4	6	
SECONDARY	40	Kiburu	Njabini	Tulaga	54.05
		12	18	10	
POST-SECONDARY	19	Kiburu	Njabini	Tulaga	25.68
		5	9	5	

Factors affecting choice of pesticide

Factor	**No of Respondents**	**%**
Increased yield	30	48.4
Cost effectiveness	20	32.3
Suggested by agrochemical Dealer	3	4.8
Suggested by other farmers	6	9.7
Good for the environment	3	4.8

Effect of the Pesticide on the environment

	Aware	Not Aware
No of People	53	21

Training on pesticide management & safety

		FARMERS	CHEMICAL DEALERS	EXTENTION OFFICERS
1	Training on pesticide management	30	6	4
2	Use of protection when handling pesticides	50	5	4
3	Information on pesticide related pollution	0	0	0
4	Suffering from any illness related to pollution	0	0	0

5	Knowledge of bodies that control use of pesticides	35	8	4

Rating of pesticides used.

	RATE	FARMERS	CHEMICAL DEALERS	EXTENSION OFFICERS
1	Poor	42	4	-
2	Fair	9	3	3
3	Good	10	1	1
4	Very Good	1	-	-

Training sessions for use of pesticides for farmers as stated by extension officers and chemical dealers

FREQUENCY		EXTENSION OFFICERS	FARMERS	CHEMICAL DEALERS
Monthly		–	-	–
Annually		1	5	1
Others	on demand (when need arises they organize, field days, exhibitions and trade fairs)	3	39	2
	When introducing new products in the market.	-	18	5

APPENDIX IV

Table A3: Physical and chemical properties of Metalaxyl M

Pure active ingredient: purity of 97.4% (S-enantiomer included), 97.2% (R-enantiomer).

Appearance	it is pale yellow in color, clear and viscous liquid
Smell	has a weak odour
Boiling point	(thermal decomposition) of 270^0 C (lower than bp)
Relative density	1.125 at 20^0C
Vapour pressure	$3.3\text{x}10^{-3}$ pa at 25^0C
Henry's law constant	$3.5\ \text{x}10^{-5}\text{pa.m}^3\text{/mol}$
Solubility in water	26g/l at 25^0C
Constant of dissociation in water	No dissociation
Partition coefficient in Octanol/water	log p_{ow} =1.17±0.04 at 25^0C
Hydrolysis (sterile solution)	stable up to pH 7 and even at 50^0C at pH 9 Has 116 days Half-life at 250C and its half–life at 500C is 7.7 days and at 600Cis 2.7 days
Photolysis in water	Light do no degrade Metalaxyl-M (solution of 2.2 ppm and pH 7, irradiated for 240h). Sunlight of wavelength above 290 nm is not absorbed by Metalaxyl-M, therefore degradation in the surface water by direct photolysis of Metalaxyl-M is not expected

Technical grade (97.1% ai)

How it appear	Its Light brown in color, viscous and clear liquid.
Smell	It has a weak odour.
Min purity	FAO has not specified for Metalaxyl-M Metalaxyl: specified by FAO as 950g/kg minimum
Melting point	71.8-72.3^{0}C
Impurities present	It may contain a maximum 1g/kg of 2, 6-dimethylaniline

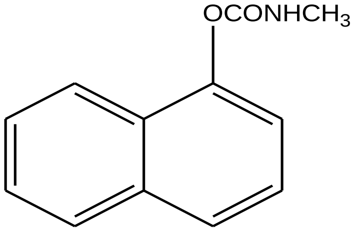
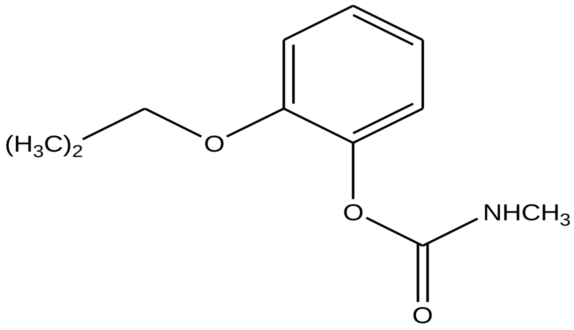
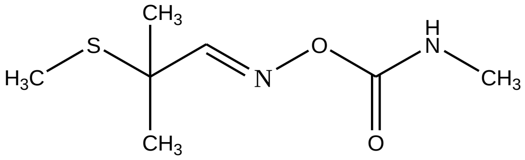
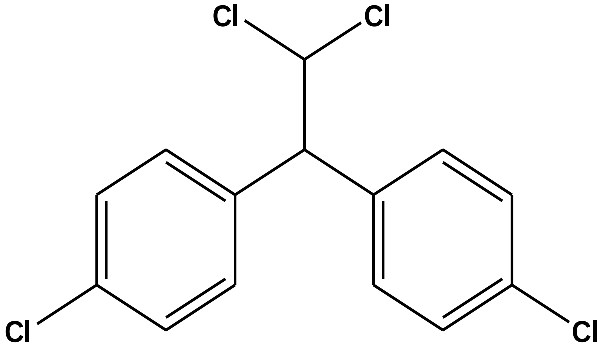
Appendix V

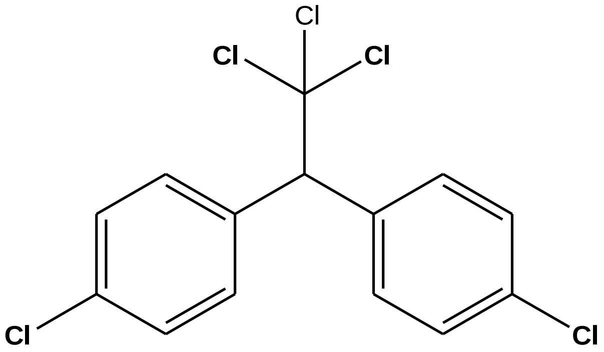
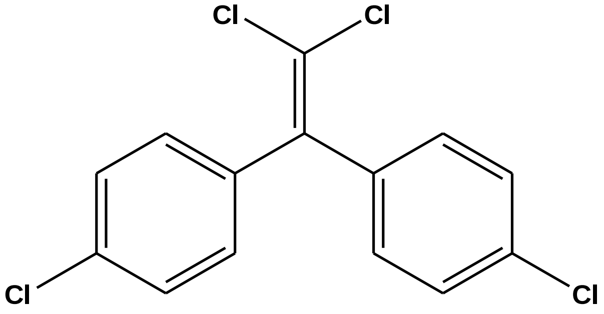
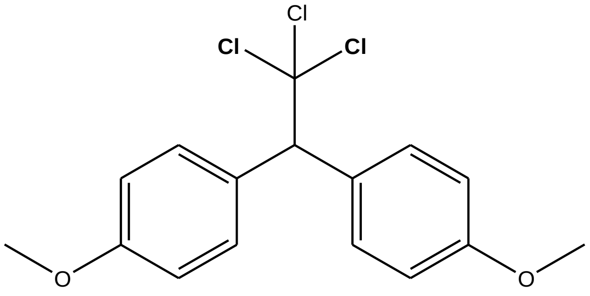
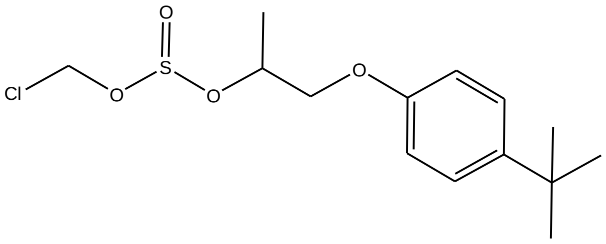
Table A4: Physical and chemical properties of Thiamethoxam

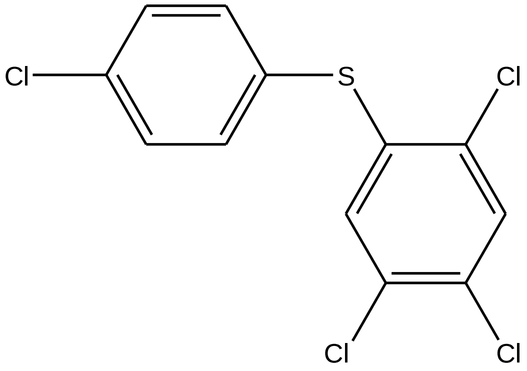
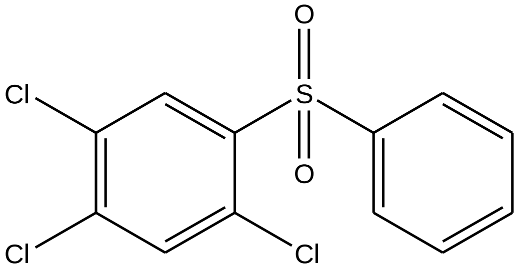
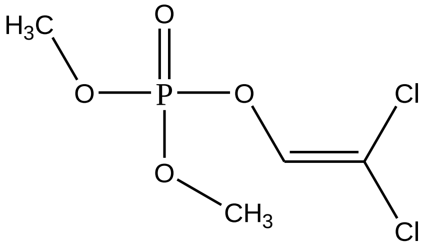
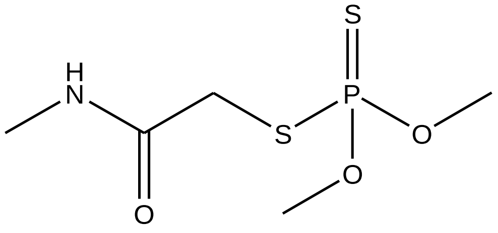
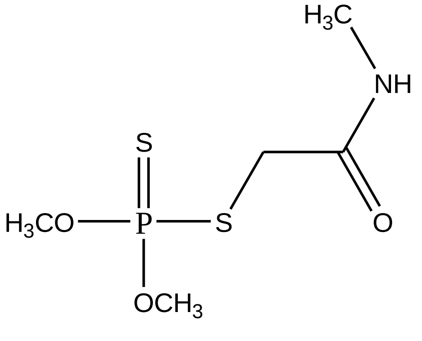
Its melting point (m.p)	Has m.p of 139.1 ^{0}C (412.3k) (its purity: 99.7%)
Its boiling point (b.p)	It starts to decompose (thermo) at about 1470C (i.e before its b.p is reached) (purity: 99.3%).
Temperature of decomposition	Thermo decomposition at about 147^{0}C.
How it appear	Fine crystalline powder slightly cream in colour (99.7% purity).
Relative density	At 200C has R.D of 1.57x103 kg/m3 which corresponding R.D of 1.57 (purity 99.7%).
Solubility of Thiamethoxam in water (g/l or mg/l)	pH 7: 4.1g/l at 25^{0}C.
Solubility in organic solvents (in g/l or mg/l)	Acetone 4.8g/l, ethyl acetate 7.0 g/l, Dichloromethane 110g/l, Hexane < 1mg/l, Toluene: 680mg/l, Methanol: 13 g/l, n-octanol 620mg/l

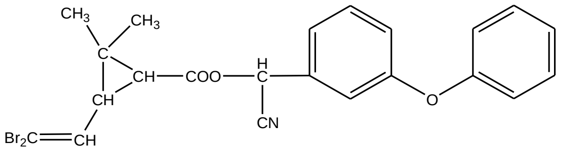
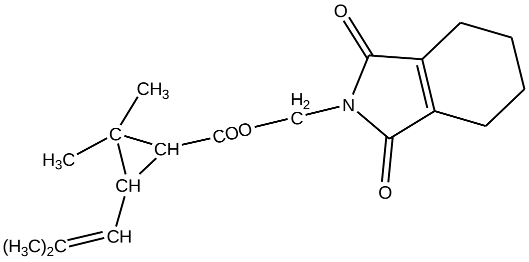
APPENDIX VI

Table A5: Structures of different types of Pesticides

Type		Name	Structure
Carbamate	1	Carbaryl	
	2	Carbofurans	
	3	Aldicarbs	
Oganochlorine	1	(1,1-dichloro-2,2-bis chlorophenyl)(4-ethane)p,p DDD	

	2	Dichloro-diphenyl-trichloro-ethane(pp-DDT)	
	3	2,2-bis p-chlorophenyl 1,1-dichloroethylene-p,p-DDE	
	4	Methoxychlor	
Organosulfur	1	Aramite	

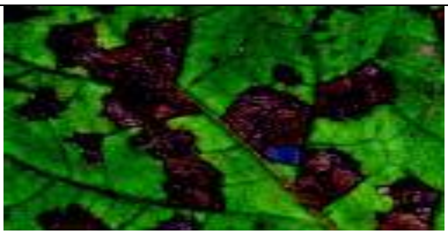
	2	Tetrasulf	
	3	Tetradifon	
Organophosphorus	1	Dichlos	
	2	Dimethoate	
	3	Diazinon	

Pyrethroids	1	Deltmethrin	
	2	Tetramethrin	

Appendix VII

Table A6: MAJOR SNOW PEAS AND POTATO PEST AND DISEASES.

S/No	Name	Picture
MAJOR SNOW PEAS DISEASES.		
1	Ascochyta blight *(Ascochyta* spp.)	
2	Downy mildew (Peronospora viciae)	
3	Powdery mildew (*Erysiphe pisi*)	
MAJOR SNOW PEAS INSECTS		
1	Cluster caterpillar (*Spodoptera litura*)	

2	Two-spotted mite (*Tetranychus urticae*)	
3	Heliothis (Helicoverpa armigera and Helicoverpa punctigera)	
Major Potato Diseases		
1	Late Blight (Phytophthora infestans)	
2	Powdery Mildew (Erysiphe cichoracearum)	
3	Early Blight (Alternaria solani)	
Major Potato Insects		
1	Leafminer Flies	

2	Blister Beetles (*Epicauta* spp.)	
3	Leaf Beetles (*Diabrotica* spp)	

Appendix VIII

Published paper

© 2017 IJSRSET | Volume 3 | Issue 5 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section Science and Technology

Dissipation Studies of Metalaxyl M Pesticide on Potatoes In Nyandarua County, Kenya

[1]**Bonface N. Kamau, Shem O. Wandiga**[1,2] **and Vincent O. Madadi**[1]

[1]Department of Chemistry, School of Physical Sciences, College of Biological University of Nairobi, P.O. Box 30197-00100, Nairobi.

[2]Institute of Climate Change and Adaptation, College of Biological University of Nairobi, P.O. Box 30197-00100, Nairobi.

ABSTRACT

Vegetables are widely consumed because of the vital role they play to provide vitamins and minerals in the diet, besides supplying proteins and energy. However they are prone of attacks by various pests and diseases leading to high loses and poor quality products. The overall objective of this study was to assess whether dissipation of Metalaxyl M on potatoes in Nyandarua County was contributing to ineffective management of the crops evidenced by high loses of the crop produce irrespective of continuous application of the pesticide. We collected samples from the farms and markets analysed resides of Metalaxyl M using GC-MS. The half-life of Metalaxyl M dissipation was calculated using the Langmuir-Hinshelwood kinetic model. The results revealed Metalaxyl M dissipation half-life of 3.13 days and 3.61 days on potatoes and soil respectively. The residues of Metalaxyl M were below the limit of detection after the 7th day of application suggesting that the pesticide was no longer effective early and late bright. All the Potatoes samples collected from the three markets open air of Njabini, Kiburu and Tulaga had no residues of Metalaxyl M. Lack of integrating data on dissipation of Metalaxyl M pesticide in developing application intervals of the pesticide is contributing to ineffective control of crop pests and diseases in production of potatoes in the region.

Keywords: Metalaxyl M dissipation, pesticides half-lives, crop pests and disease protection.

I. INTRODUCTION

Horticulture sector has grown in the last few decades to become one of the major foreign exchange earner in many developing countries. In Kenya the major crops include fruits, vegetables and cut flowers production.1 Inaddition, agricultural sector, mainly livestock husbandry and crop production, accounts for about 25% of Kenya's GDP with an estimated 75% of the population depending on the sector either directly or indirectly.2 Among the crops, Irish potato (Solanumtuberosum) is one of the most common vegetables grown by farmers in Kenya.3 They have a wide variety of uses, for instance they can be consumed when fried, boiled, roasted or mashed.3 They are a good source of nutrition in that they are rich in carbohydrates, potassium, fiber and Vitamin C.4 In 2012, yield was 2.9 million tonnes which had a value of 50 billion Kenya shillings.2 The main potato growing areas in Kenya are Nyandarua, Nakuru, Meru and Bungoma counties.2

Chemicals contamination in food has been described as a potential risk to human health, including the development of chronic diseases.5 Food is the main exposure route and exposure to pesticide residues through the diet is assumed to be five orders of magnitude higher compared to other routes such as air and drinking water.6 Because fruits and vegetables are mainly consumed raw or semi-processed, it is expected that they contain higher residue levels compared to other food groups of plant origin, such as bread and other processed foods. Given the potential risk of pesticides for public health, the use of pesticides in fruits and vegetable production is subjected to constant monitoring.7 Pesticides maximum residues levels were established to ensure food safety. The MRLs are always set far below levels considered to be safe for humans.8,9

In 2005, Kenyan passion fruit and French beans were intercepted in Sweden and Belgium during routine

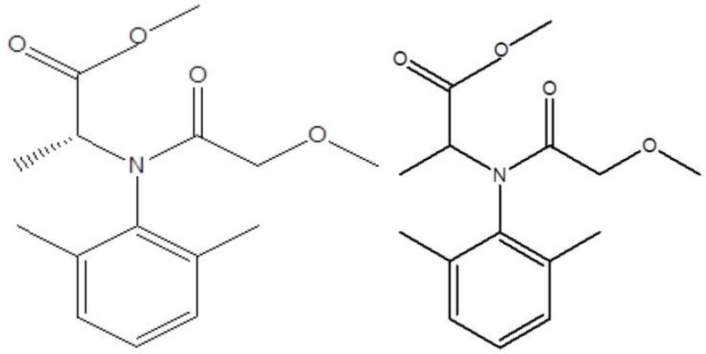
IJSRSET173460 | Received : 13 July 2017 | Accepted : 23 July- 2017 | July-August-2017 [(3)5: 221-229]

monitoring. The passion fruits were found to have dithiocarbamates and Chlorothalonil residues, while the French beans were found to have Chlorothalonil, Dimethoate, Tebuconazole and Omethoate residues.10

A study on dissipation and degradation of Malathion and Dimethoate from soil and garden pea plant was carried out by Getanga11 revealed foliar dissipation rate of Dimethoate from garden pea of 29 days (t1/2 =29) while the foliar dissipation rate of Malathion from the pea plant was fast during the initial stages and slowed afterwards.

Metalaxyl-M is a systemic fungicide for foliar, soil and seed treatment. It is widely used for the treatment of potato bright (photophthora) and penospora in vines. It is used on vegetables, peas, hops, citrus, other fruits and tobacco. It is often sold in combination with protectant fungicides such as mancozeb and copper fungicides. Metalaxyl is a recemic mixture of two isomers, one of which, the R-enantiomer is the active form. This enantiomer is the basis of the fungicide, Metalaxyl-M (Mefenoxam), which is effective at half the application rate of Metalaxyl. The toxicological profile of Metalaxyl and Metalaxyl-M was evaluated in the framework of Directive 91/414/EEC, which resulted in an acceptable daily intake (ADI) and an acute reference dose (ARfD) being established at 0.08 mg/kg bw per d and 0.5 mg/kg bw, respectively. The MRL proposals were derived from the use of Metalaxyl-M but it cannot be excluded that the use of Metalaxyl leads to higher residue levels than those observed for Metalaxyl-M.12

Indiscriminate use of systematic fungicides especially Metalaxyl (Ridomil) provides chance to develop resistance strain of the fungus. Metalaxyl resistant strain of P infectans has been reported in Bangladesh.13 Phenyl amide fungicides are a class of systematic compounds including Metalaxyl (ridomil oxadxyl benalaxyl and ofurace) that show excellent protective, curative and eradicative antifungal activity and exclusively control diseases caused by peronospoles.14 An increase of Metalaxyl – resistant isolates of phytophtora infestans was observed between 1981 and 1984, the period during which phenyl amide fungicides were suspended in Ireland and the Netherlands. The active ingredient of Ridomil Gold is Metalaxyl M which is shown in Figure 1.



(a) Metalaxyl M Methyl N-(Methoxyacetyl)-N-(2,6-xylyl)-D-alaninate

(b) Metalaxyl Methyl N-(Methoxyacetyl)-N-(2,6-xylyl)-S-alaninate

Figure 1. Chemical structures of Metalaxyl M and Metalaxyl

A study carried out in Estonia for 3 consecutive years 2003, 2004 and 2005 showed that the increase of frequency of Metalaxyl- resistant isolates was detected. Metalaxyl – resistant isolate were less frequent among isolates collected in 2003 than in 2004 and 2005. Metalaxyl –resistant isolates was found to fluctuate from year to year due to the fact that the quality of Metalaxyl resistant isolates had increased compared with previous results.15 The reason for the increase could be the more consistent with the regular use of fungicides containing

Metalaxyl. As long as Metalaxyl is used according to the recommendations it is unlikely that Metalaxyl –resistant isolates will completely displace sensitive isolates.15 They noted that inspite of the occurrence of resistance strains the use of fungicides consisting of Metalaxyl could be used effectively for control of potato late bright at maximum of two times at the beginning of the fungicide treatment. By following the application rules for Metalaxyl fungicides it is possible to restrict development of Metalaxyl – resistant strains thus avoid the reduction of efficacy of the fungicide. 15

The overall objective of this study was to conduct dissipation studies of Metalaxyl M on potatoes and to investigate the levels of Ridomil Gold pesticide residues in market products of potatoes in Nyandarua County.

II. MATERIALS AND METHODS

A. Study area:

In Nyandarua County, potato farming is a venture that is helping the small scale farmers to boost their income. However, the yields are profoundly affected by fungi and pest such as insects. Pesticides are of great benefit to agriculture in Kenya due to decreasing quality and quantity of crop production due to insects, weeds, plant diseases, rodents and other pests. Furthermore, in the recent past farmers complained of ineffecetiveness of Ridomil Gold against fungi on potatoes.

B. Materials:

Anhydrous sodium sulphate, Na_2SO_4 for use was prepared by backing it for 16 hours at 200 ^{0}C in the oven to remove all the impurities. SiO_2 (1.8g per sample) was dried overnight at 200 ^{0}C to make it 100% active (remove all water). Deactivation of SiO_2 with water: SiO_2 (1.5%, w/w) was carried out by adding 1.5 ml of HPLC grade water to 98.5 g of activated SiO_2 in a 250 ml Erlenmeyer flask and shaken by hand until all lumps were eliminated. The Erlenmeyer flask was placed on a shaking platform for half an hour. After deactivation, these chemicals were left overnight to condition. The performance of SiO_2 was tested before use with real samples.

Copper powder was activated by shaking with 9 ml of dilute hydrochloric acid solution (3:1 ration for H_2O: HCl). The mixture was centrifuged for 1 minute at 300 rpm and acid solution discarded. The powder was rinsed three times with methanol to eliminate all the hydrochloric acid, and the supernatant discarded. The remaining powder was dried under a gentle stream of white spot nitrogen before use.

C. Sample Preparation:

The laboratory samples of potato leaves, stems, roots and tuber were homogenized using Hobart food processor. The homogenized wet analytical sample (10 g) was placed in a motor. For every gram of wet sample, 3g of baked out anhydrous Na_2SO_4 was added. This mixture was ground using a pestle to a homogeneous powder. This was then covered with aluminium foil and left overnight to dry further. This was done in triplicate.
The dry sample for extraction was put in a thimble made of filter paper and placed in a Soxhlet. Solvents hexane and acetone in the ratio of 1:1 were placed in a 250 ml round bottomed flask and the Soxhlet extraction was set in triplicate for each sample and was allowed to extract the sample for 16 hours. Three replicate samples of (5 g each) of the homogenized wet sample were weighed in a weighing glass and were kept in an oven to dry for moisture content determination. After drying the dry mass was re-weighed and the moisture content was determined from the two masses. This was repeated for all the samples extracted.

For soil extraction, 20 g was weighed into a beaker and ground with Na_2SO_4 added in the ratio of 1 g of sample to 2 g of Na_2SO_4. The ground sample was covered with aluminium foil and left overnight to dry further. Soxhlet extraction was set as described above and allowed to extract for 16 hours. In all cases anti-bumping chips were added to allow smooth boiling. The extract was rotary evaporated (concentrated) to 1ml using rotary evaporator and then taken for clean-up process.

Sample clean-up was done using silica. A chromatographic clean-up column 20 cm length and 2 cm internal diameter (with frit) was packed with anhydrous Na_2SO_4 to 1 cm height (equivalent to 2 g) and 1.8 g of SiO_2 followed by 0.5g of activated charcoal and another 1 cm of Na_2SO_4. After every addition, the column was tapped to allow the particles to settle uniformly. The column was then conditioned by eluting with 15 ml of hexane: acetone mixture (ratio 1:1) and discarded.

The Soxhlet extract of the sample was transferred quantitatively to the column. The tube was further rinsed with five 1 ml portions of hexane: acetone mixture and the rinsing transferred to the column as soon as the sample extract had eluted without leaving the column to dry completely. The column was then eluted with 165 ml of hexane: acetone mixture (ratio 1:1). The eluant was collected in a 250 ml round bottomed flask. Eluant was rotary-evaporated to 1.0 ml. The extract was transferred to a GC sample vial (auto vial) and further concentrated to 0.5 ml using a gentle stream of nitrogen. The extract was now ready for analysis by GC- MS for Metalaxyl M.

To remove sulphur from the cleaned soil extracts, approximately 1g of freshly activated copper powder was added to the sample in a vial. A black coloring appeared to all extracts containing sulphur which indicated a formation of copper sulphide compound. The extracts were then filtered through glass funnel which was packed with glass wool and activated 2g anhydrous sodium sulphate. 5 ml of HPLC hexane: acetone mixture (ratio 1:1) was used to condition the anhydrous sodium sulphate and the sample was introduced and eluted with 20 ml of HPLC grade hexane: acetone mixture (1:1) into round bottom flask and concentrated to 3 ml using rotary evaporator. The concentrated samples were transferred to clean auto vials and then concentrated to 0.5 ml under a gentle stream of white spot nitrogen gas and taken for GC-MS analysis for Metalaxyl M.

Analysis of the pesticide residues was carried out using Agilent 6890 GC equipped with 5972-2 MSD avavailable at the University of Nairobi, Department of Chemistry pesticide laboratory. DB-5 fused silica capillary column of 30 m x 0.25mm i.d. x 0.25µm film thickness was used for separation. The carrier gas was helium N6 with 99.99% purity, at a flow rate of 1.0 ml/min. Oven temperature was maintained initially at 70 ^{0}C for 1 min, increased at 15 ^{0}C/min to 175 ^{0}C, then at 2 ^{0}C/min to 215 ^{0}C, at 10 ^{0}C/min to 265 ^{0}C and finally at 20 ^{0}C/min to 290 ^{0}C and held for 8 minutes. Injection volume was 1µL, in splitless mode at injection temperature of 250 ^{0}C.

D. Quality Assurance and Quality Control:

Control pots for dissipation of Metalaxyl M in potatoes and in soil were set alongside the experimental pots. The control pots were separated to exclude any contamination from the treated pots. Analysis of controls was carried out alongside the samples to ascertain that no artifact in the crop derived from local conditions gave rise to interference in the analysis and to establish the transport and storage stability of any residue. Field blanks consisting of distilled anhydrous Na2SO4 were carried along at every field trip to track field contamination and were then subjected to the entire analytical procedure as the samples. Extraction was carried out in triplicate.

E. Moisture Content Determination:

Moisture content in plant and soil samples was determined by heating 5 g of the sample in pre-cleaned and pre-weighed watch glass in an oven (model E 28# 04-71528) at 105oC for 24 hours. The difference in weight between wet and cool dry sample was taken as the moisture content. Percentage moisture was calculated as:

$$\% \text{ moisture} = \frac{\text{Moisture content}}{\text{Weight of wet sample}} \text{ x } 100$$

F. Statistical Data Analysis:

The data obtained was analyzed using Statistical Programme for Social Scientists (SPSS) and Microsoft Excel programme. Calculation of half-life was done using Langmuir rate equations. Results are presented as mean of triplicate analysis with standard deviation.

III. RESULTS AND DISCUSSION

A. Method accuracy and Recovery Tests:

Recovery data for pesticide residue analysis method were generated by fortifying blank samples with 50 µl of 1 mg/kg Metalaxyl M. These were then analysed to establish the recovery of the analyte. The cleanup stage was optimised and gave recovery of 105.30 % for Metalaxyl M with elution of 165 mls hexane:acetone (ratio 1:1), 95 % for Metalaxyl M with elution of 165 ml of hexane. 165 ml of hexane:acetone mixture (ratio 1:1) was therefore used as eluting solvent for clean up. Potatoes, soil and blank (unhydrous Na2SO4) samples were spiked with 50 µl of 1 mg/kg Metalaxyl M and the percentage recoveries calculated. The recoveries of Metalaxyl M ranged between 75.15 % and 92.95 %. The recovery of Metalaxyl M was found to be 86.50 % in soil, 75.15 % in potatoes and 92.95 % in blank samples respectively (Table 1). The recoveries were within the acceptable range of 70 % - 120 %.

Table 1: Method Recovery, data in % for Metalaxyl M

Sample type	Metalaxyl M
Potatoes	75.15±2.3
Soil	86.50±2.8
Control	92.95±5.4

B. Quantification Tests:

Quantification was based on calculations from calibration curve for Metalaxyl M. The calibration curve of the standard was linear with R2 of 0.99. Figure1 below shows calibration curve for Metalaxyl M. Concentrations of analytes were obtained using the equation: Y= mx+c. Where Y = Peak area (Instrument response); x = Analyte concentration ; m = gradient ; and c = Constant.

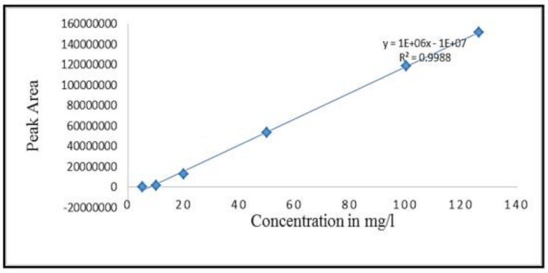
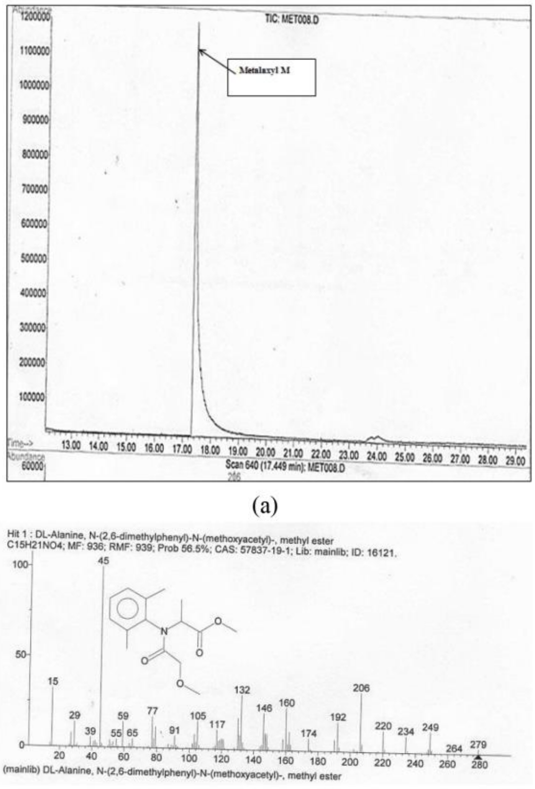


Figure 2: Calibration curve of Metalaxyl-M standard

The ion chromatogram of metalaxyl M is shown in Figure 2 below. The average retion time was 14.45 minutes.



(b)

Figure 3 : Ion chromatogram and mass spectrum of Metalaxyl M standard. (a) Ion chromatogram (b) mass spectrum

C. Dissipation of Metalaxyl M in potatoes:

The spray mixture applied to the potatoes contained guarantee Metalaxyl M 40 g/kg of Ridomil Gold. The potatoes (matures in 90 days) were treated once with Metalaxyl M to determine the effectiveness of a single application. Samples were collected on day 0, 2, 4, 7, 14, 21 and 28 and for residues of Metalaxyl M. The results obtained are presented in Table 2.

Table 2 : Results of Metalaxyl M Dissipation in Potatoes in mg/Kg (Dry weight).

Day	Treated Potatoes	Untreated Potatoes
0	9.01±0.52	<0.05
2	4.05±0.24	<0.05
4	3.45±0.02	<0.05
7	1.72±0.06	<0.05
14	<0.05	<0.05
21	<0.05	<0.05
28	<0.05	<0.05

The residues of Metalaxyl M were below the limit of detection after the 7th day of application suggesting that after that period, the crops were nolonger protected agaianst early and late bright.

The dissipation behavior of Metalaxyl M is shown in Figure 5 obtained by plotting determined concentration of Metalaxyl M against time in days. The concentration of Metalaxyl M in potatoes decreased over time. The average initial deposition of Metalaxyl M was 9.01±0.52 mg Kg−1 (day 0) and the final residue was 1.72±0.06 mg Kg−1 on day 7. After 2 days 44.95 % of Metalaxyl M remained in the potatoes while after 4 days 38.29 % of the initial deposited pesticide remained in the potatoes and 19.09 % of the initial deposited Metalaxyl M remained in the plant by day 7. No residue was detected after day 7. The results showed that there was rapid dissipation of Metalaxyl M in the potatoes for the first 2 days and that the characteristic two-phase dissipation pattern showing the initial faster dissipation rate followed by slower rate starting after 2 days was seen.

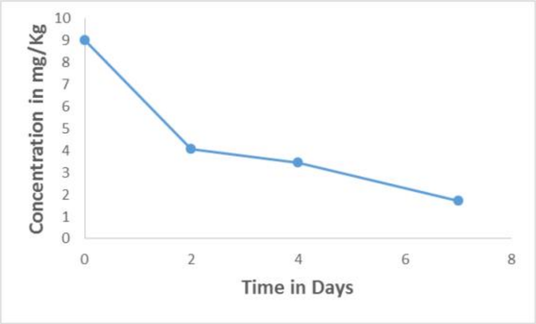


Figure 3: Trends of Metalaxyl M Dissipation in Potatoes

The data was fitted into Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration.[16]

$R = dC/dt = kKC/(1+KC)$---------------(1)

Where R is the rate of reaction (mol/L.min), C is the equilibrium concentration of reagent (mol/ L), t is the time (min), k is the rate constant (1/min), and K is the Langmuir constant (L/mol). When the initial concentration Co is <<<1, the denominator in equation 1 above can be ignored as 1, and the equation can be simplified to an apparent first-order equation:

$dC/dt=kKC$---------------------------- ---(2)

$dC/C=kKdt$---------------------------------(3)

$dC/dt = -Kt$--------------------(4)

Integrating equation 3 and taking boundary conditions of C = Co at t = 0, gives

$Ln(Co/Ct) = kKt = k_{abs}$---------------------(5)

Or

$Ct= Coe^{-kt}$ -- --- ------(6)

This is the first order rate equation, also written as:

$\ln C_t = \ln(C_0) - K_{obs}Xt$------------(7)

Where; C_t= pesticide concentration at time, t; K_{obs} = first order rate constant; t= time in days; and C_o = the original Metalaxyl M concentration.

Consider the half-life of the reaction where the remaining concentration of the pesticide is half the original amount; $C_t= C_o/2$ and substituting in equation 7 above gives:

$Ln(C_o/2C_o=-Kt_{1/2}$-------------------------- (8)

$Ln\ 0.5=-Kt_{1/2}$---------------------------- -- (9)

$-0.693/K =t_{1/2}$----------------------------- (10)

The regression curve for the disappearance of Metalaxyl M is shown in Figure 6 which was obtained by plotting a graph of negative logarithm of the concentration of residues against time and it had correlation of R^2 =0.9483.

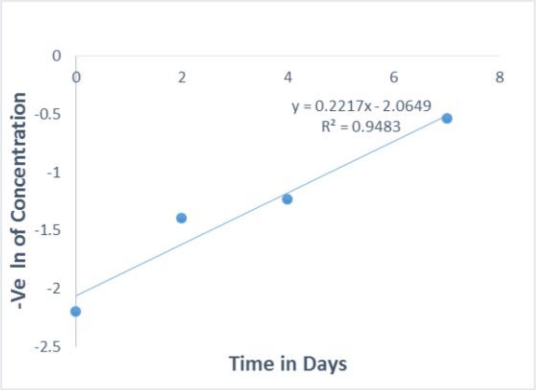


Figure 4 : Regression curve for disappearance of Metalaxyl M from Potatoes

Equation 7 is in the form of y = mX + C. A plot of ln (C_t) verses time (t) gives a straight line, the slope of which upon linear regression equals the apparent first-order rate constant $K_{obs.}$ In this case, the K_{obs} are the observed degradation rate constant. The values obtained by this analysis were the same as that of the exponential regression analysis. Based on first order kinetic, a plot of negative ln concentration of residues versus time t (days) Figure 6, gave a regression equation, y = 0.2217x-2.0649. A gradient of 0.2217 was obtained (which is equivalent to the constant K_{obs}). In this study the degradation of Metalaxyl m follows Langmuir-Hinshelwood kinetic equation and using equation 10, the half-life of Metalaxyl M in Potatoes was 3.13 days by the Langmuir-Hinshelwood kinetic model.

D. Dissipation of Metalaxyl M in soil:

This study was conducted to establish whether application of Metalaxyl M on crops growing on treated soil resulted in elevated residue. The results are presented in Table 3.

Table 3: Results of Metalaxyl M Dissipation in Soil (mg/Kg, dw)

Day	Treated Soil	Untreated Soil
0	5.03±0.25	<0.05
2	2.52±0.01	<0.05
4	1.91±0.09	<0.05

7	1.23±0.02	<0.05
14	<0.05	<0.05
21	<0.05	<0.05
28	<0.05	<0.05

Figure 7 below shows Metalaxyl M dissipation in soil, with fastest rate experienced during the first two days but later declines over time. The average initial deposition of Metalaxyl M was 5.03±0.25 mg Kg^{-1} (day 0) and the final residue was 1.23±0.02 on day 7. The rapid dissipation of Metalaxyl M in soil during the first 2 days followed by lower rate revealed a two-phase dissipation pattern.

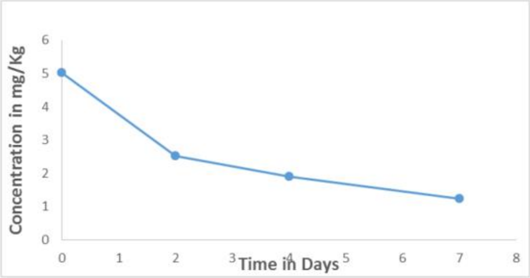


Figure 5: Trends of Metalaxyl M Dissipation in Soil

The data fitted well into Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration.[16] The regression curve for the disappearance of Metalaxyl M in soil is shown in Figure 8 below obtained by plotting a graph of negative logarithm of the concentration of the residues against time. A correlation of R^2 =0.9456 was obtained.

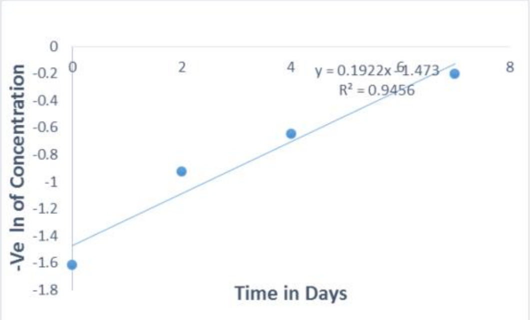


Figure 6 : Regression curve for disappearance of Metalaxyl M from Soil

The half-life of Metalaxyl M in soil was calculated using equation 10 and data obtain (Table 3). The half-life (t1/2) of Metalaxyl M was calculated through regression analysis assuming the loss of Metalaxyl M follows Langmuir-Hinshelwood kinetic model, after k was determined (gradient = 0.1922) and was found to be 3.61 days.

E. Assessment of Metalaxyl M Residues in Potatoes:

Potato samples from three open air markets of Njabini, Kiburu and Tulaga were analysed for Metalaxyl M residues. The results presented in Table 4 below show that Metalaxyl M residues were not detected in all potatoes from the three markets surveyed. Potatoes sold in the three markets had residue levels below the European Union limit of 0.05 mg/Kg.

Table 4 : Assessment of Metalaxyl M Residues in Potatoes

Season	Njabini	Kiburu	Tulaga
Wet (October-November)	<0.05	<0.05	<0.05
Dry (January-March)	<0.05	<0.05	<0.05

F. Discussion of results:

The average initial concentration of Metalaxyl M was 5.03±0.25 mg/kg on day 0 and decreased to 1.23±0.02 mg/Kg by the 7th day. The dissipation curve for the pesticide showed that the dissipation of the residues has initial fast rate for the first two days followed by a slower rate. The half-life was found to be t1/2 = 3.61 days. The amount of residues at harvest depends upon factors such as the rate of application, time interval between last application and harvesting, and the type of crop. Other factors include environmental factors such as temperature, precipitation and humidity and air movement (wind) which influence the persistence of pesticides in plants. The total quantity and form of pesticide reaching plants and soil depends on the site and method of application, type of equipment together with the formulation type. These consequently influence pesticide persistence on both the plants and soil.17, 18,19 Plant factors that affect persistence include plant species, nature of harvested crop, structure of cuticle and metabolic activities like rate of uptake, growth, translocation storage and excretion.17 In this study the observed trend may be attributed to environmental factors due to high precipitation and low temperatures experienced in Kinangop Sub-County.

IV. CONCLUSION AND RECOMMENDATION

There are major pests' problems in Kinangop Sub-County and therefore an active pest management programme is necessary to secure the harvest in the region. Metalaxyl M pesticide did not exhibit persistence in both soil and potatoes. The half-life was 3.13 days and 3.61 days in Potatoes and soil respectively. Metalaxyl M residues after day 7 were below the limit of detection, clearly indicating that after day 7 the pesticide is not effective protection of the crops against early and late bright in potatoes. The concentration of Metalaxyl M residues in all potato samples collected from the three open air markets of Njabini, Kiburu and Tulaga was below detection limit. Since Metalaxyl M residues were below detection limit within 7 days of applicationson potatoes, farmers should be advised to incorporate data on dissipation of the pesticide for effective control against early and late bright. Metalaxyl M pesticide was not detected in all market samples analysed indicating that the potatoes are safe for consumption if they are sprayed at recommended dosage and harvested at normal post harvesting interval of 14 days. More sampling times is suggested for study of dissipation rate on single and multiple application of Metalaxyl M to investigate residual effects under different seasons and application modes. National Pesticide Residues Monitoring and Surveillance is recommended on produce sold in the Kenyan markets and those destined for export to protect consumers and control misuse of pesticides to support safe trade.

V. REFERENCES

[1]. Export Processing Zones Authority (EPZA), (2005) Report on Horticulture Industry in Kenya, pp 9. Nairobi, Kenya.

[2]. Horticultural Crops Development Authority (HCDA), (2013) Horticulture Validated Report 2012. United States Agency for International Development (USAID) and Ministry of Agriculture (MOA), Nairobi, Kenya.

[3]. Youth Agro-Environment Initiative (YAEI), 2012. Small scale potatoes growing business in Kenya, 2012. Available online at http://yagrein.blogspot.com/2012 /05/small-scale-potatoesgrowing-business.html. Accessed: November 2015.

[4]. Muraguri, J., Mutwiri,K., Agawo, D., Arjan, M., Mbogo, S., Juma, V. and Muli, J., (2012) Kenya Agricultural Research Institute stunning success in potato breeding. Hortfresh Journal. Dec-Feb 2012: pp. 20-21.

[5]. Souza M. V. and Caldas E.D., (2006) Residues of Dithiocarbamates and organophosphorus Pesticides in Food for a Vegetarian Meal and Non- Vegetarian Diet in Brasilia, Brazil. 11th IUPAC International Congress of Pesticide Chemistry. Book of Abstract (2) for Poster Session. Port Island, Kobe, Japan pp 209.

[6]. Juraske, R., C. mutel, F. stoessel and S. Hell weg, (2009) Life Cycle human toxicity assessment of pesticides: Comparing fruit and vegetable diets in Switzerland and the United States. Chemosphere, 77:939-945.

[7]. Claeys, W., S. Jean-Francois, C. Bragard, G. Maghuin-Rogister, pLuc and B. schiffers, (2011) Exposure of several Belgian Consumer groups to pesticide residues through fresh fruit and vegetable consumption. Food cont., 22:508-516.

[8]. EFSA, (2010) 2008 Annual report 0n pesticide residues according to article 32 of regulation (EG) No396/2005. EFSA journal 8(6), 1646.

[9]. OECD (2010) OECD Survey on pesticide maximum residue limit (MRL) policies: survey results. OECD series on pesticides, No. 51.

[10]. Kenya Plant Inspectorate services (KEPHIS), (2006) Annual Report, July 2005-June 2006; Nairobi, Kenya.

[11]. Getenga Z. M., Jondiko J. I. O., Wandiga S. O. and Beck E., (2000) The Dissipation behaviour of Malathion and dimethoate from the garden pea plant (Pisum sativa). Bull. Environ. Contam. Toxicol. 64, 339 - 367.

[12]. EFSA (European Food Safety Authority), (2015) Reasoned opinion on combined review of the existing maximum residue levels (MRLs) for the active substances metalaxyl and metalaxyl-M. EFSA Journal 13(4):4076, 56 pp. doi:10.2903/ j.efsa.2015.4076.

[13]. Dey, T, K. and M. S. Ali, (1994) Pathological research on tuber crops in Bangladesh. In: proc of workshop on transf. of Tech of CDP crops under Res. Ext linkage program, held on oct.22-27 BARI GAZIPUL Bangladesh. pp 159-165.

[14]. Schwinn. F.J & staub, T., (1987) Phynylamide and other fungicides against Oomycetes.in lyr. H. (ed): modern selective fungicides. Jena: VEB Gustar Fischer, pp 259-73.

[15]. Runno, E., & Koppel, M., (2006) The question of metalaxyl resistance on late blight fungus in Estonia. Agronomy Research (Special issue): 341-344.

[16]. Kar R., Oindrila G., Kunal M., and Sangita B. (2013). Performance Study on Photocatalysis of Phenol Solution in a UV Irradiated Reactor. J. Chem. Eng. Process Technol. 3, 4:1.

[17]. Edwards C.A., (1975) Factors that Affect the Persistence of Pesticides in Plants and Soils. Rothamsted Experimental Station, Harpenden Herts., UK.

[18]. Musila S., (2011) Fate of Chlorothalonil on snowpeas and assessment of its residues in French Beans and Passion Fruits in Nairobi. M.sc. Thesis, Department of Chemistry, University of Nairobi, Nairobi, Kenya.

[19]. Bempah, C. K., A.K Donkor, P.O. Yeboah, B. Dubey and P. Osei-Fosu, (2011) A preliminary assessment of consumer's exposure to organochlorine pesticides in fruits and vegetables and the potential health risk in Accra metropolis, Ghana. Food chem, 128:1058-1065.