

**PESTICIDE RESIDUES IN SOME VEGETABLES ROTATED WITH
TOBACCO USING HPLC, AND FARMERS' AWARENESS OF
PESTICIDE HEALTH EFFECTS IN KURIA- MIGORI, KENYA**

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**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for any degree in any other university.

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DEDICATION

This work is dedicated to my loving husband Mr. Lucas Oyugi, our lovely daughters Lucia and Angela and sons Antony, Martin and Francis who gave me all the support and inspiration.

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ABBREVIATIONS AND ACRONYMS

ACfD	Acute reference Dose
ADI	Acceptable Daily Intake
a.i	Active Ingredient
ANOVA	Analysis Of Variance
BAM	Behaviour Assessment Model
BchE	Butyrlcholinesterase
CPMRA	Canada's Pesticide Management Regulatory Agency
DAD	Diode Array Detector
EFSA	European Food Safety Authority
EU	European Union
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FSSAI	Food Safety and Standard Authority of India
GAP	Good Agricultural Practices
GC	Gas Chromatography
GC-MS	Gas Chromatography -Mass spectrometry
GC-MS-MS	Gas Chromatography - Mass spectrometry - Mass spectrometry
GPC	Gel Permeation Chromatography
GWP	Groundwater Potential
HPLC	High Performance Liquid Chromatography
ICSC	International Chemical Safety Cards
IUPAC	International Union of Pure and Applied Chemistry

LC-MS	Liquid Chromatograph- Mass spectrometer
LC ₅₀	Lethal Concentration that kills 50% of the animals in a test population
LD ₅₀	Lethal Dose that kills 50% of the animals in a test Population
MP	Mobile Phase
MRLs	Maximum Residue Limits
MSPD	Matrix Solid Phase Dispersion
NPIC	National Pesticide Information Centre
OP	Organophosphates
PMRA	Pesticide Management Regulatory Agency
PRC	Pesticide Residue Committee
RfD	Reference Dose
RUP	Restricted Use Pesticide
SP	Stationary Phase
SPSS	Statistical program for social scientists
TCP	Trichloropyridinol
USEPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture
USGAO	United States General Accounting Office
UV	Ultra Violet
v.p	Vapor Pressure
WHO	World Health Organization

ABSTRACT

Pesticides bring a promise of higher yields of more food for the hungry and freedom from diseases spread by pests. However, pesticides are poisons and their residues do harm to human beings when the concentration is higher than the recommended maximum residue limits (MRLs). If a pesticide is left on vegetables, which are considered a cheaper source of natural nutrients, somebody could get sick or even get killed due to the poisoning. Therefore pesticide residues have become a high concern issue in the field of food safety. Tobacco being a pesticide dependent crop requires different types of pesticides in the control of pests; these include methomyl (N-methylcarbamate) and acephate (organophosphate). These pesticides are known to be very toxic. They act directly on the nervous system by inhibiting the neurotransmitter acetylcholine in addition to many other effects. Due to limited land resource and high levels of poverty, the same pieces of land used for planting tobacco are also used for planting other food crops like vegetables. There is a possibility that these pesticides may also be used to control pests in the vegetables or may get into the vegetables during spraying of tobacco. However very few reports are available on the levels of these pesticides in vegetables in Kenya. Thus, there is need to determine the levels of the pesticide residues in the vegetables, and assess the farmers' awareness of the health effects of these pesticides. Therefore this study assessed farmers' awareness of the health effects of pesticides and the use of protective measures. The levels of methomyl and acephate pesticide residues were determined in selected vegetables that were planted on the same soil where tobacco had been harvested without further application of the pesticides in Kuria West district, Migori County of Kenya. Vegetables were also sprayed directly with methomyl and acephate pesticides after planting and analyzed for the same. Questionnaires were pretested and thereafter distributed and then analyzed. Samples were collected from both the farms and seedbeds used for tobacco during wet and dry seasons. A total of 273 samples were collected during the months of Jan to May 2010 and analyzed. The pesticide residues were extracted with ethyl acetate and analyzed by high performance liquid chromatography (HPLC). The data was analyzed using t-test, and ANOVA. The study established that 74.6% of those interviewed were aware of the effects of the pesticides; however 81.4% did not wear protective clothing when using the pesticides. The levels for acephate ranged between 0.0030 ± 0.0000 mg/Kg to 0.1570 ± 0.0430 mg/Kg with kales having the highest followed by amaranthus. Some samples had values that exceeded the minimum residue limit (MRL) (0.03 mg/Kg) recommended by European Food Safety Authority (EFSA), World Health Organization (WHO) and Food and Agriculture Organization (FAO). The levels for methomyl ranged between 0.0010 ± 0.0003 mg/Kg and 0.0001 ± 0.0000 mg/Kg with all the samples having values below the minimum residue limit MRL (0.05 mg/Kg) recommended by World Health Organization (WHO) and Food and Agriculture Organization (FAO). Pesticide levels were found to be significantly higher than the recommended levels for acephate during the dry season as compared to the wet season. The results obtained by this study will be availed to relevant authorities for appropriate action and could also be used to sensitize farmers.

CHAPTER ONE

1 INTRODUCTION

1.1 Background information

Most pesticides and chemicals are not biodegradable, and due to bioaccumulation, can enter into food chain and ultimately affect human and animal health. Environmental exposure of pesticides to humans through ecosystems may be during cropping, agriculture practices, and consumption of food materials or air inhalation. According to Yadav (2010), without pesticides or agricultural chemicals, crop yield could drop by as much as a third and food prices would increase by as much as 75%.

Pesticides are known to circulate constantly in the biosphere migrating from one environmental object to another remaining in each of them for sometime. They penetrate all links of animate nature and objects of living nature. Pesticide exposure can cause many different health effects from acute problems such as dermatitis and asthma to chronic problems such as chronic obstructive pulmonary disease and cancer (Sanborn *et al.*, 2002).

Since most pesticides are toxic in nature, their continuous ingestion by man even in trace amounts can result in accumulation in body tissues with serious adverse health effects (Handa *et al.*, 1999). Carbamate and organophosphorus pesticides have been reported to be potential acetylcholinesterase inhibitors (California department of pesticide regulation, 1998). Inhibition of acetyl cholinesterase, an

enzyme used in muscle relaxation, results in buildup of acetylcholine, which in turn, causes continuous and excessive muscle stimulation leading to tetany, paralysis and eventual mortality (Boettger and McClintock, 2010).

Carbamate insecticides are widely used in agriculture and gardening. They are derivatives of carbamic acid. Their mechanism of action is that of inhibiting the vital enzyme acetylcholinesterase. They can also cause metabolic acidosis however; respiratory depression combined with pulmonary edema is the usual cause of death from poisoning by N-methylcarbamate compounds (Agha *et al.*, 2009).

Organophosphorus pesticides are a class of compounds that include derivatives of phosphoric, phosphorus, thiono-phosphoric and thion-thiolo-phosphoric acids esterified with methyl or ethyl and different alcohol groups. Depending on the alcohol moiety, the polarity of organophosphorus pesticides range from water soluble compounds such as dimethoate to lipophilic compounds such as bromophos-ethyl. Majority of organophosphorus pesticides are insecticides with anti-cholinesterase activity. Depending mainly on the chemical stability and dose, time and mode of application, therefore pesticide residues may occur in crops and derived products (Di muccio *et al.*, 2006).

The behavior of pesticides in the agricultural produce is of great importance, since the disappearance, persistence, or partial transformations of such compounds

determine their usefulness or their potential effects to our environment (Bergmann *et al.*, 1989, Wang and Liu, 2001, Chang *et al.*, 2005).

Tobacco has received attention mainly due to the damage smoking habit causes to human health. Only recently other specific problems not related to tobacco consumption itself have been looked into by society and government entities linked to health and environment. Such problems are related to impacts of pesticides to the environment and human health caused by tobacco farming.

Most people are aware that tobacco consumption has significant effects on health, including an increased risk of lung cancer and cardiovascular disease. However people may be less aware of the problems associated with widespread application of pesticides used in growing tobacco (Ghelli *et al.*, 2009). Unfortunately up to now, only a few studies addressing these problems have been reported (Hermes *et al.*, 2007).

Tobacco is a pesticide dependant crop as its broad and succulent leaves provide favourable conditions for the development of many pests and diseases (Khan *et al.*, 2008). The culture practice adopted by industries in tobacco farming comprises of periods of seedling production (seed beds), land preparation, seedling transplantation, harvesting, curing and drying. Seedling production and land preparation steps involve the use of the greatest quantity of agro-chemicals, fertilizers and pesticides (Hermes *et al.*, 2007). After transplanting the seedlings, some farmers utilize the fertilized soil in the seedbeds for the production of human

foods including kales, spinach, amaranth, tomatoes, cabbages, maize, beans, and others. Figure 1.1 shows a farmer weeding vegetables planted on a seedbed that had been used for tobacco, next to tobacco seedlings awaiting transplantation.



Figure 1.1: Farmer weeding a seedbed of vegetables on a former tobacco seedbed and next is a tobacco seedbed

Most of the pesticides used on tobacco are also widely used on food and other crops, as well as in residential settings. Specifically, 1997 survey data estimated that about 27 million pounds of the 37 pesticides were used on tobacco, while the estimated use of these pesticides nationally on all crops was 175 million pounds (USGAO, 2003).

Tobacco is widely planted in many regions in Kenya, among them Kuria West District, Migori County in Nyanza province, where it is extensively planted as a cash crop.

1.2 Pesticides on vegetables

Vegetables are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. Like other crops vegetables are attacked by pests and diseases during production and storage leading to damages that reduce the quality and yield. In order to reduce the loss and maintain quality harvest, pesticides are used together with other pest management techniques during cropping to destroy pests and prevent diseases (Keikotthaile and Spanoghe, 2011).

Use of pesticides during vegetable production often leads to the presence of pesticide residues in them after harvest. The presence of pesticide residues is a concern for consumers because pesticides are known to have potential harmful effects to other non-targeted organisms than pests and diseases (Gilden *et al.*, 2010). Some of the pesticides are persistent and therefore remain in the body causing long term exposure.

Several studies carried out in developed and developing countries have pointed out food contamination due to presence of pesticide residues in the environment (Anderson and Poulsen, 2001; Sanghi and Tewari, 2001; Sanghi and Sasi, 2001).

Leamy and Evans (2009) reported that some vegetables and fruits could have nine different pesticides in a single serving, and that kales are among the dirty dozen because they require a lot of pesticides to grow. The results from studies discussed above indicate that, there is a possibility of the vegetables produced from soils used for tobacco being contaminated with pesticide residues, and therefore need to analyze them on regular basis.

1.3 Problem statement and justification

Vegetables play an important role in human nutrition. Apart from ascorbic acid (vitamin c) which is the principle vitamin supplied by fruits and vegetables, leafy vegetables are a rich source of essential nutrients. Therefore it is necessary for farmers to use pesticides to control pests and diseases that attack vegetables for better yield. However pesticides are toxic, and result in a number of health effects on those that apply them and also the consumers who eat the vegetables that contain high levels of pesticide residues. Farmers may use higher amount of pesticide than recommended quantities to kill the pests not wait for the stipulated period of time before harvesting the vegetables (after application of the pesticides). There is also a possibility that the farmers are not aware of the side effects of the pesticides and the protective measures. Acephate and methomyl are some of the pesticides used in tobacco growing and due to limited land resources and high levels of poverty, farmers plant other food crops on the same soils after tobacco and in some cases intercrop some food crops with tobacco. This study therefore determined the levels of acephate and methomyl pesticide residues in selected

vegetables; (amaranth (*Amaranthus retroflexus L.*), kales (*Brassica oleracea L.*) and spinach (*Spinacia oleracea L.*) planted on soils after tobacco which is a pesticide dependant and a major cash crop in Kuria West District had been planted. The study investigated the effect of spraying the vegetables directly with acephate and methomyl pesticides on the residue levels and assessed farmers' awareness of pesticide health effects from two divisions of Kuria west district.

1.4 Hypotheses

- i) Farmers spray vegetables directly with pesticides used for tobacco and are not aware of the side effects and protective measures.
- ii) Kale (*Brassica oleracea L.*), spinach (*Spinacia oleracea L.*) and amaranth (*Amaranthus retroflexus L.*) planted on the same soils after tobacco do not contain methomyl and acephate pesticide residues.
- iii) Kales, spinach and amaranth planted on the same soil after tobacco and sprayed with methomyl and acephate do not contain high levels of the pesticides residues.

1.5 Objectives

1.5.1 General objective

To determine levels of some pesticide residues and their reduction rates in selected vegetables planted after tobacco, and to assess farmer's awareness of pesticides side effects and protective measures from the pesticides.

1.5.2 Specific objectives

- i) To assess farmers' awareness of the side effects of methomyl and acephate pesticides and the use of these pesticides on the vegetables.
- ii) To determine levels of methomyl and acephate pesticide residue in amaranth, kales and spinach planted on the soils used for tobacco growing with no further application of the pesticides, during both the dry and wet seasons.
- iii) To determine levels of methomyl and acephate pesticide residues in amaranth, kales and spinach planted on the soil used for tobacco and the effect of spraying with these pesticides, during both the dry and wet seasons.
- iv) To determine the reduction rate of acephate and methomyl in kales planted on soils on which tobacco seedlings had been grown.

1.6 Significance of the study

This study was able to come up with valuable information on the levels of methomyl and acephate pesticides residues in the vegetables planted after tobacco. The study also assessed farmers' awareness of the side effects of the pesticides used. The results will be availed to the policy makers who may use it to come up with policies on the use of pesticides and also to sensitize the public on pesticide residues in vegetables and consequent repercussions.

1.7 Scope and limitations of the study

This study was conducted in two out of four divisions of Kuria West district due to limited funds. The divisions were Mabera and Kehancha. The study analyzed

acephate and methomyl pesticide residues in amaranth (*Amaranthus retroflexus L.*), kales (*Brassica oleracea L.*) and spinach (*Spinacia oleracea L.*) but their metabolites were not considered. Soil analysis was not done before planting of the vegetables. Only kales were used in the experimental garden at Kenyatta University, and harvesting of sprayed kale was done from day 2 up to 15 days as per the recommended period (USDA, 1995). The samples were collected both during the wet and dry seasons. The vegetables were sampled from the farm and market stations.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Pesticides

Pesticides are manmade and naturally occurring chemical substances used to control insects, plants and fungal pests in agricultural, domestic and institutional settings. Pesticides can be grouped by their target organisms (Raven *et al.*, 1997). The main groups of commonly used pesticides include herbicides, insecticides, fungicides, fumigants and rodenticides (Sanborn *et al.*, 2002).

Pesticides are widely used throughout the world in agriculture to protect crops. However, they are not only toxic to target organisms but also to non-target organisms and could have harmful effects to animals and man. The widespread use of pesticides pollutes our environment and threatens public health. Pesticides are among the priority pollutants to be monitored in a wide variety of matrices due to their incorporation into foods, waters and soil, which may represent a potential hazard to human health (Chuanjiang *et al.*, 2010).

Insecticides generally are the most toxic pesticides to the environment, followed by fungicides and herbicides (Yadav, 2010). Exceptions exist for certain herbicides which are highly toxic, and are far more hazardous to the environment than are insecticides (Yadav, 2010). The most hazardous pesticides include those that can be distinguished on the basis of either water or fat solubility. Water soluble compounds are easily transported out of the target area into ground water

and streams; fat soluble chemicals are readily absorbed in insects, fish, and other animals, often resulting in extended persistence in food chains. The amount of pesticide residue that remains reflects, among other things, the amount of pesticide applied, the time lapsed since application, and the speed with which the pesticide dissipates in the environment. Residue levels remaining on crops are also affected by where the pesticides are applied, such as in the soil or on the plant, and when they are used in the life cycle of the plant, such as when the plant is a seedling or shortly before the plant is harvested (USGAO, 2003).

Intake of an excessive amount of pesticides can lead to acute intoxication while longtime exposure can cause chronic poisoning. Many pesticides have also been identified as potential carcinogens (Patton, 2006). The most serious pesticide poisoning usually result from acute exposure to organophosphate and carbamate insecticides (Lorenz, 2006).

Several pesticides including organophosphorus and carbamate insecticides have been linked to severe health conditions in humans including neurological damage, hypertension, cardiovascular diseases and skin disorders (Wesseling *et al.*, 2001; Snodgrass, 2001; Calvert *et al.*, 2001; Turusor *et al.*, 2002; Tchounwou *et al.*, 2002). Some pesticides have been found to cause infertility and sterility, and birth defects. Others have been linked to allergies, hematologic disorders, mutagenicity and cancer (Raminez and Cuenca, 2001; Payne *et al.*, 2001; Tchounwou *et al.*, 2002). Toxicity and bioaccumulation studies of the insecticides with the trade name 'Raid' in Wister rats, showed that animal mortality progressively increased

with increasing concentrations while growth in weight decreased (Achudume *et al.*, 2008). There is a danger of phytotoxicity when pesticide levels accumulate in the plant (Waller, 1987).

Tobacco ranks sixth behind potatoes, tomatoes, citrus, grapes and apples in the amount of pesticides used per acre (USGAO, 2003; Ghelli *et al.*, 2009). Studies conducted in Sawabi district, Pakistan, showed that tobacco farmers had multiple pesticide residues above acceptable daily intake in their blood and this decreased the activity of butyrylcholinesterase (BchE) in the farmers exposed to the pesticides (Khan *et al.*, 2008). Different countries have set residue limit for some of the pesticides used in tobacco in both green and cured tobacco, examples including Germany, Spain and Italy. Table 2.1 shows Residue Limits Adopted by Germany, Italy, and Spain for some of the Pesticides.

Table 2.1: Residue Limits Adopted by Germany, Italy, and Spain for some of the pesticides

Pesticide	Germany ^a	Italy ^b	Spain ^c
Acephate	d	1.5	d
Aldicarb	10.0	0.6 (green) 3.0 (cured)	5.0
Benefin	d	0.01	0.02
Carbaryl	3.0	3.0	0.1
Carbofuran	20.0	0.1	10.0
Chlorpyrifos	d	0.2	0.05
Diazinon	1.0	d	0.02
Malathion	3.0	0.5	0.5
Maleic hydrazide	80.0	80.0 ^a	80.0
Mancozeb	50.0 ^c	2.0 (green) 10.0 ^f (cured)	0.05 ^g
Metalaxyl	d	1.0	3.0
Methomyl	2.0	d	d
Methyl bromide	20.0	d	d

Source; US GAO (2003)

Note: GAO's analysis of CORESTA and European Court of Auditors data.

a-Residue limit on finished products.

b-Residue limit on green tobacco if not otherwise specified.

c-Residue limit on dried tobacco.

d-Country has not adopted limits for this pesticide.

e-Residue limit established for the entire class of dithiocarbamates except metam.

f-Residue limit established for the entire class of dithiocarbamates except thiram.

g-Residue limit established for the entire class of dithiocarbamates except metam and thiram.

Most of the pesticides used on tobacco are widely used on food and other crops, as well as in residential and other settings. Table 2.2 shows pesticides commonly used on tobacco as reported by the general accounting office in the United States (USGAO, 2003).

Table 2.2: Pesticides Commonly Used on Tobacco, 1990-98

Primary use(s)	Pesticide
Insecticide	Acephate, aldicarb, <i>Bacillus thuringiensis</i> , carbaryl, carbofuran, chlorpyrifos, diazinon, disulfoton, endosulfan, ethoprop, fenamiphos, fonofos, imidacloprid, malathion, methidathion, methomyl, spinosad, trichlorfon
Herbicide	Benefin, clomazone, diphenamid, isopropalin, napropamide, pebulate, pendimethalin, sethoxydim, sulfentrazone
Fungicide	Dimethomorph, mancozeb, mephenoxam, metalaxyl
Plant growth regulator	Ethephon, flumetralin
Plant growth regulator, herbicide	Maleic hydrazide
Fumigant, insecticide	Chloropicrin
Fumigant, insecticide, herbicide	Methyl bromide
Fungicide, insecticide, herbicide	1, 3-dichloropropene (1,3-D)

Source; USGAO (2003)

A study by Patton (2006) showed that 70% of the 55 samples of vegetables studied contained pesticide residues with 30% (17 samples) exceeding international standards. Another study in Belgium reported that 1413 samples of fresh or frozen fruits and vegetables analyzed showed that 28.2% had no residues, 6.1% exceeded the minimum residue limit (MRL) set in regulation, with more exceeding levels observed in foodstuffs from the third countries than in products from the European Union (Pesticides residue monitoring in food of plant origin Belgium, 2008). Anderson *et al.* (1999) reported that out of 3438 surveillance samples of fruits, vegetables, juices, fruit drinks, cereal grains, vegetable oils, cereal products and oil

seeds analyzed for residues of 202 pesticides, 73 samples (2.1%) exceeded the National and EU harmonized MRLs.

According to Hanak *et al.* (2002) surveys carried out by institutions spread throughout China indicated that 50-70% of vegetables are contaminated with insecticide residues. This necessitates continuous analysis of pesticide residues in all foods and especially those consumed locally.

Analysis of pesticide residues in grapes from India by the pesticide residues committee (PRC, 2004) reported two samples containing methomyl at 0.07 mg/kg (MRL 0.05 mg/kg) and acephate at 0.04 mg/kg (MRL 0.03 mg/kg). In Kuria West Kenya, these pesticides are used in tobacco and vegetable farming, which fall under organophosphorus and the carbamate pesticides.

2.2 Organophosphorus pesticides

Organophosphorus pesticides are a closely related group of compounds that affect the function of the nervous system in humans. They are used on many food crops, in residential and commercial buildings and for ornamental plants and lawn care which means many people may be exposed to them through different routes. One of the most important routes is dietary exposure (Salvador *et al.*, 2006). While they break down quickly in the environment and do not accumulate in body tissues, organophosphate pesticides are much more acutely toxic to humans and animals

than the persistent organochlorine pesticides which have largely been replaced (USGAO, 2003).

Organophosphates also have acute psychological and behavioral effects such as anxiety, depression and cognitive impairment (Mearus *et al.*, 2006). Epidemiological studies conclude that acute and chronic organophosphate exposure is associated with effective disorders (London *et al.*, 2005). Ecological studies and case series also support a causal association between organophosphates use and suicide (London *et al.*, 2005). Therefore, organophosphates are not only agents for suicide; they may be agents of causal pathway (London *et al.*, 2005). An example of organophosphate insecticide commonly used is acephate.

2.2.1 Acephate

Acephate is an organophosphate foliar spray insecticide of moderate persistence with residual systematic activity of about 10-15 days at the recommended rate (USDA, 1995). The IUPAC name for acephate is O, S – Dimethylphosphoramidothioate, molecular formula is $C_4H_{10}NO_3PS$, and the molecular structure is shown in Figure 2.1. It is commonly known as orthene (98% acephate).

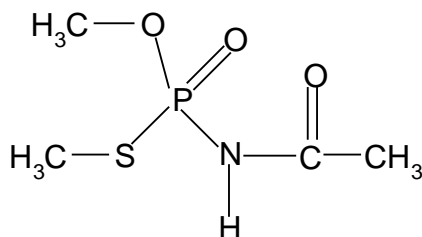


Figure 2.1: Structure of acephate

The LD₅₀ for mammals is medium (LD₅₀ = 500 - 5000 mg/kg) to high (LD₅₀ = 50 - 500 mg/kg), and acute toxicity from inhalation is lethal at LC₅₀ = 2-20 mg/l (USDA, 1995). Acephate is considered a fetotoxin and there is some evidence of hormonal effects (USDA, 1995).

2.2.2 The toxicological effects of acephate

Exposure effects of acephate to humans can include cardiac responses, central nervous system impairment, eye problem, gastrointestinal problems, respiratory effects and death due to respiratory failure (USDA, 1995). The maximum residue limit for acephate is 0.03 ppm (PMRA, 2008).

A study using the behavior assessment model (BAM) and the groundwater potential model (GWP) assessed the contamination of groundwater by acephate and methamidophos. It was observed that acephate had a longer half-life than methamidophos in soil, but the mobility of methamidophos in both aerobic and anaerobic soils was slower than acephate, leading to the conclusion that acephate may lead to the contamination of groundwater much more easily than methamidophos under normal conditions (Yen *et al.*, 2000).

Another study on dissipation of acephate, in green mustard and top soils carried out in 2009 reported that acephate dissipation followed first order kinetics with half life of between 1.1 and 3.1 days in green mustard and between 1.4 and 9.4 days in top soils (26°C), with higher vapor pressure of insecticides and higher rainfall appearing to stimulate dissipation from the vegetables (Chai *et al.*, 2009). It was observed that methamidophos derived from acephate amounted to less than 10% by mass of the parent compounds in soils, hence concluding that vegetable shading possibly retarded pesticide degradation in soil (Chai *et al.*, 2009). Another report by Li (2005) showed that the degradation of acephate increased the concentration of methamidophos residues when the insecticide was sprayed on vegetables.

According to Chuanjiang *et al.* (2010), acephate applied at concentrations (36 or 72 g a.i l⁻¹) could result in high methamidophos residue levels on pakchoi and vegetables even after an 18 days interval between sampling and spraying. Reports on acephate residues in vegetables in Kenya are lacking.

2.3 Carbamate pesticides

Carbamate pesticides kill insects in a similar fashion as organophosphorus pesticides. They are widely used in homes, gardens and agriculture. Most carbamates are extremely toxic to hymenoptera, and precautions must be taken to avoid exposure to foraging bees or parasitic wasps. Some of the carbamates are

translocated within plants, making them an effective systematic treatment (Fishel, 2008).

Carbamate pesticides are reversible acetyl cholinesterase inhibitors, which have shorter duration of action (Ryan *et al.*, 1999). Their principle route of entry is either by inhalation or ingestion or secondarily by the dermal route. They are hydrolyzed enzymatically by the liver, degradation products excreted by the kidney and liver. Respiratory depression combined with pulmonary edema is the usual cause of death from poisoning by carbamate compounds (Fishel, 2008). Examples of carbamate pesticides include methomyl, aldicarb, carbaryl, methiocarb, oxamyl, thiocarb, carbofuran and fenoxycarb.

2.3.1 Methomyl

Methomyl is a carbamate insecticide that is widely used for the control of a large range of insects. It has been classified as a pesticide of category -1 toxicity (Patil *et al.*, 2008). It is a member of the N-methylcarbamate class of chemicals. The IUPAC name is S-methyl-N-(methylcarbamoyl) thioacetimidate. The molecular structure is shown in Figure 2.2. It is commonly known as lannate (90% methomyl).

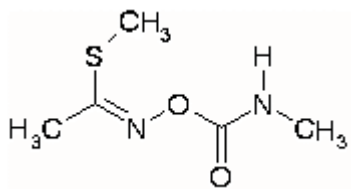


Figure 2.2: Structure of methomyl

The N-methylcarbamates can leave residues that can be detrimental to food safety even when applied in accordance with good agricultural practices (GAP) (Chang *et al.*, 2007). The primary route of human exposure to methomyl is consumption of contaminated food or water from contaminated wells.

2.3.2 The toxicological effects of methomyl

Methomyl treated rats showed histopathologic changes in kidney and spleen of male and female rats. It also influences mixed function oxidase and creates abnormality of liver functions in rats, this depends on the dose and duration of methomyl (Patil *et al.*, 2008). Studies revealed that this pesticide may pose a ground water contamination risk, because it was found beneath the application area at 90 cm depth (Kahl *et al.*, 2007). The maximum residue limit for *Brassica* vegetables is 0.05 mg/kg (FAO, 2002).

Methomyl is considered very toxic to mammals, and is classified by the EPA as a restricted-use pesticide (RUP), or class I. Reported LD₅₀ values are 17-24 mg/kg in rats, 10 mg/kg in mice and 15 mg/kg in guinea pigs. It is moderately highly toxic to fish with reported LD₅₀ values of 3.4 mg/L for rainbow trout exposed for 96 hrs

and 0.8 mg/L for bluegill sunfish. It is also highly toxic to aquatic invertebrates with $LD_{50} = 0.0287$ mg/L for *Daphnia magna* (Farre *et al.*, 2002).

Methomyl is a highly toxic N-methylcarbamate with (Z) - and (E) - isomers and used in agricultural pesticides with lethal dose (LD_{50}) of less than 50 mg/kg. Several fatal cases of accidental and suicidal poisoning with methomyl have been reported in literature with central nervous system (CNS) depression being most common cause. Methomyl intoxication is also associated with development of acute pancreatitis and various case reports have proposed oxidative injury mechanism as a possible explanation of the acute pancreatitis (Agha *et al.*, 2009).

The degradation of methomyl in 0.001% $KMnO_4$, was 48%, in 0.9% NaCl was 39%, in 0.1% $NaHCO_3$ was 43% while in 0.1% acetic acid was 43% (Klinhom *et al.*, 2008). This was attributed to the difference in behavior and physiochemical properties in pesticides but the effectiveness of the washing solutions on carbamate pesticide removal is still unknown (Klinhom *et al.*, 2008).

2.4 Vegetables

Vegetables are considered as 'protective supplementary foods' as they contain large quantities of minerals, vitamins and essential amino acids, which are required for normal functioning of the metabolic processes. They are important to neutralize the acids produced during digestion and also useful as 'roughage' (Shanmugavelu, 1989).

Pests, diseases and weeds can take their toll on vegetables in terms of quality and quantity. Some pests for vegetables are insects and closely related species like butterflies, weevils and maggots. There are currently many pesticides which are used to control pests, diseases and weeds many of which are poisonous to other organisms besides the targeted ones. The production of vegetables ranges from large scale farm enterprises and market gardens growing for profit to private gardens or homesteads, where vegetables are an essential element of the families own efforts to supplement their diet or income.

2.4.1 Kales (*Brassica oleracea L.*)

Kale grows well in strong, moist soil that is enriched with organic matter. However, it will grow in nearly all soil types provided that drainage is satisfactory (Rodriguez, 2010). Tall varieties of kale are transplanted when they are ten to fifteen centimeters (four to six inches) high. Kale rarely suffers from pests, but when it does it is subject to the same pests as cabbage: cabbage worms, cabbage loopers, harlequin bugs, root maggots, cut worms, aphids, thrips and flea beetles (Rodriguez, 2010). The whole plant can be harvested at once or extend the harvest by picking individual leaves. Kale can be harvested at any time as soon as the leaves are large enough (21-28 days). Kale leaves are best eaten when young, because they can get a bit tough when older, but are still perfectly edible.

Kales and collard greens are very nutritious and have powerful anti oxidant properties and are anti-inflammatory. These greens have very high carotene, vitamin

k, vitamin C, zeaxanthin and reasonably rich in calcium. Kales also contain folic acid, folate, and folacin (a B-complex) vitamin known for its importance in pregnancy and prevention of pregnancy defects (Ukwendu, 2009). Kale and cruciferous vegetables more generally have been shown to inhibit the growth of human pancreatic cancer cells and to reduce the risk of occurrence of lung, gallbladder, and bladder, prostate, ovarian and colorectal cancer. Kale generally is considered resistant to pests, hence many people think that it contains low levels of pesticide residues. However a study by Sadja (2011) reported high pesticide residues in kales.

Another study by Rerkasem (2004) reported that in Thailand Chinese kale was among commodities of high pesticide residue with 20% of the samples examined found to exceed FAO's allowable maximum residue level. Njagi (2007) also reported that kales from urban areas in Kenya had much higher mean residue levels of deltamethrin (0.2 mg/kg) compared to kales from rural areas which had 0.12 mg/kg. From these studies and many others it is evident that kales are likely to contain many pesticide residues.

2.4.2 Spinach (*Spinacia oleracea* L.)

Spinach is a leafy vegetable with dark green roughly triangular leaves. The plant is smaller and has a lower growing rate than garden or silver beet, but serves much the same purpose (Langer and Hill, 1991). Spinach reaches edible maturity quickly (37 to 45 days) and thrives best during the cool, moist seasons of the year. During periods of warm weather and long days, spinach will produce seed (Sanders,

2001). Spinach can be grown successfully on a variety of soils, but a fertile sandy loam high in organic matter is preferred (Sanders, 2001). Spinach is ready for use as soon as it is edible size and it must be harvested before there is extensive yellowing, breakage and other leaf deterioration or the development of seed-stalks (Sanders, 2001). Aphid and leafminer are the two predominant insect pests of spinach which are controlled by pesticides among them acephate and methomyl.

Spinach is a source of carotene (provitamin A), ascorbic acid and minerals as well as being a good source of thiamin and riboflavin (Edmond *et al.*, 1990). Often referred to as the “power food”, spinach is packed with essential nutrients such as calcium, iron and vitamins. Unfortunately it is not appreciated by many who regard it as a foul tasting vegetable that they are urged to eat for reasons of health. Spinach is believed to have originated in Iran but today it is cultivated and eaten all over the world (Shapley, 2010). It grows in temperate climates. There are two varieties of spinach, distinguishable by the fact that one is the curly leaf variety while the other is the flat leaf variety. The flat leaf variety is preferred by most people as it has a milder flavor (Shapley, 2010).

Spinach contains vitamins K, E, C, B6 and A. In addition it contains manganese, folate, magnesium, iron, calcium, potassium, copper, protein, phosphorous, zinc, omega-3 fatty acids, niacin and selenium. It is a good source of dietary fiber. Eating spinach regularly provides numerous health benefits which include protection against cancer, osteoporosis, arthritis, heart disease and other diseases (Shapley, 2010).

Spinach contains several flavonoid compounds which act as anti-cancer agents (Langer and Hill, 1991). Some researchers were so impressed by the benefits of the compounds that they created spinach extracts to study their effects on various types of cancers. Their studies showed that these flavonoids slow down the division of stomach cancer cells and also reduce skin cancer (Langer and Hill, 1991). A good intake of spinach also helps to prevent colon and breast cancer (Metaljan, 2009). A carotenoid called neoxanthin that is found in green vegetables such as spinach actually induces prostrate cancer cells to self-destruct (Metaljan, 2009). Spinach is a food on which pesticide residues are frequently found. Some people may want to avoid eating spinach unless they are sure that it is grown organically.

A study by National Pesticide Information Centre (NPIC, 2011) carried out in the years 2006 and 2008 revealed that acephate residues were detected in many vegetables among them spinach. Another report by Shapley (2010) revealed that spinach can be laced with as many as 48 different pesticides making it one of the most contaminated green leafy vegetable. Also according to Young (2011) spinach is among the vegetables that 90% of samples analyzed contain pesticide residues. The Food Safety and Standards Authority of India (FSSAI, 2009) reported that spinach had the highest mean concentration of cypermethrin among all the foods analyzed in Andhra Pradesh in the year 2008. From these studies it is clear that there is a possibility of pesticide residues being present in spinach planted in rotation with tobacco.

2.4.3 Amaranthus (*Amaranthus retroflexus* L.)

Amaranthus collectively known as amaranth or pigweed is a cosmopolitan genus of annual herbs growing to height of 0.6-1m (Macrae *et al.*, 1993). Approximately 60 species are presently recognized with inflorescences and foliage ranging from purple to red to gold and include weed, grain, vegetables and ornamental types (Irving *et al.*, 1981; Kauffman and Weber, 1988). *Amaranthus* species are reported to have 30% higher proteins value than other cereals such as rice, wheat flour, oats and rye (Grubben and Denton, 2004). Amaranthus is a pseudo-cereal that has been widely grown. The small seeds contain considerable levels of high quality protein (rich in lysine) and low levels of saturated fatty acids in their oil moiety, thus winning room on the health food store shelves. In addition to its high nutritional value, amaranth grain has a hypocholestromic potential (Chatuverdi *et al.*, 1993, Plate and Areas, 2002, Berger *et al.*, 2003, Ferreira *et al.*, 2007). The vegetables described above that is, kales, spinach and amaranth, are attacked by the same type of pests, which include aphids, Colorado beetles and butterfly larvae. These pests are controlled using pesticides. Some of the studies that have reported pesticide residues in amaranthus include; a study by Khatoun *et al.*, (2004) revealed that after one day of application of cypermethrin, 0.23% of the applied dose was detected in red amaranth while 0.085% of the applied dose was detected on the 3rd day of spray. Another study by Food Safety and Standards Authority of India (FSSAI, 2009) reported that although amaranth was contaminated with a large number of pesticides, some even at high levels, the risk was lower than other foods as it is consumed in lower quantities.

2.5 Community awareness of pesticide effects

Agricultural chemicals are used heavily in farming to control pests. While all pesticides can harm humans, insecticides and herbicides cause most human poisoning (Masaya *et al.*, 2011). Pesticides affect humans through three mechanisms of entry: ingestion, inhalation and dermal absorption. In under-developed and developing countries, the least expensive pesticides are utilized due to the inability of farmers to purchase more expensive, safer products (Masaya *et al.*, 2011). Farmers, consumers as well as many officials in charge of pesticide management may be inadequately aware of the adverse effects of pesticides. As a by product of pesticide use, farmers and their families are affected daily with health problems directly resulting from pesticide exposure. Many store pesticides in the bedroom or kitchen, use pesticide containers to store drinking water, and wash pesticide-contaminated clothes together with other family clothes.

Poverty affects the pesticide problem in a variety of ways. Protective equipment is neither available nor affordable to farmers in poor countries, and moreover it is not practical to wear in tropical climates due to high heat and humidity. An illiterate farmer is unable to read the directions about proper pesticide use. Farmers' financial situation does not allow them to purchase the adequate spraying equipment or safer, but more expensive, pesticides. Yassin *et al.* (2002) reported that farm workers in the Gaza strip used pesticides extensively and despite their knowledge about the adverse health impact of the pesticides, the use of protective measures was very poor and most of the self reported toxicity symptoms occurred particularly among the younger workers.

Another study conducted by Suubashiny and Thiruchelvam (2008) reported that nearly 60% of farmers from Pampaimadu in Sri Lanka had medium level of knowledge of plant protection practices. About 6% of the farmers had good level knowledge towards the recommended plant protection measures and yet almost all farmers depended on chemical pesticides for management of pests and diseases, but at 35% higher concentrations than recommended level. According to Dalmas and Llias (2011), many users are inadequately informed about potential short and long term risks of pesticides and the necessary precautions in the correct application of such toxic chemicals are not always made. Therefore, it is critical to raise awareness and to accompany communities in making efforts to decrease exposure to agricultural chemicals.

2.6 Methods used for analysis of pesticide residues

Several methods have been used in the analysis of pesticide residues. Those used for methomyl and acephate pesticide residue analysis include; Gas chromatography with tandem mass spectrometric detection (GC-MS-MS) (Chuanjiang *et al.*, 2010), high performance liquid chromatography (HPLC) with diode array detector (DAD) and matrix solid phase dispersion (MSPD) (Wang *et al.*, 2008), liquid chromatography mass spectrometer (LC-MS) (Botitsi *et al.*, 2008). This study used HPLC with ultra violet (UV) detector because of its ability to analyze both acephate and methomyl, and also its availability.

2.7 High performance liquid chromatography (HPLC)

This is a method based on the separation of the components of a solution in the mobile phase (MP) due to differential retention of the solutes by the stationary phase (SP) (Lough and Wainer, 1996). Several detection techniques can be applied however the most commonly used is the ultra violet (UV) detectors because they are sensitive, have a wide linear range and are relatively unaffected by temperature fluctuations (Meyer, 1997; Jedlicka and Klime, 2004). The HPLC unlike GC does not require the sample to have an appreciable vapor pressure. Thus HPLC is applicable not only to the separation of compounds with lower molecular weight but also compounds with higher molecular weight (Kuwana, 1980). The conditions for HPLC are mild-usually ambient temperature with ordinary solvents such as hexane and water (Harvey and Zweig, 1980).

High performance liquid chromatography (HPLC) is a separation technique that utilizes the differences in distribution of compounds between two immiscible phases, called stationary phase and mobile phase. The stationary phase designates a thin layer created on the surface of fine particles and the mobile phase designates the liquid flowing over the particles. Under a certain dynamic condition, each component in a sample has different distribution equilibrium depending on solubility in the phases and/or molecular size. As a result, the components move at different speeds over the stationary phase and are thereby separated from each other. Figure 2.3 shows a schematic diagram of an HPLC instrument.

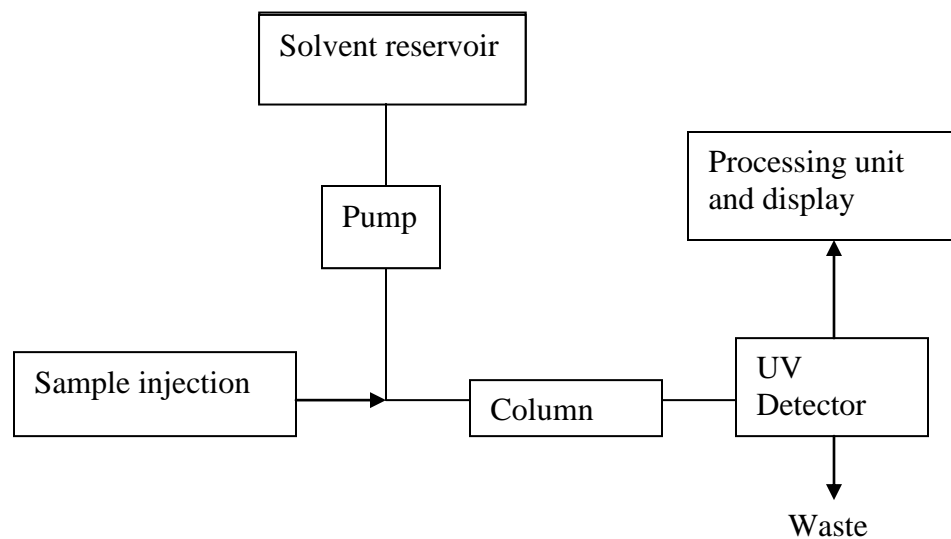


Figure 2.3: Schematic diagram of an HPLC instrument with UV detector

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Study area

The study area was Kuria West District which is located between latitudes $-1^{\circ}30'$ south and longitudes $34^{\circ}15'$ and $34^{\circ}30'$ east and is one of the tobacco growing areas in Kenya. The climate is tropical humid. Figure 3.1 shows a map of the district, divisions and the stations where samples were collected for this study.

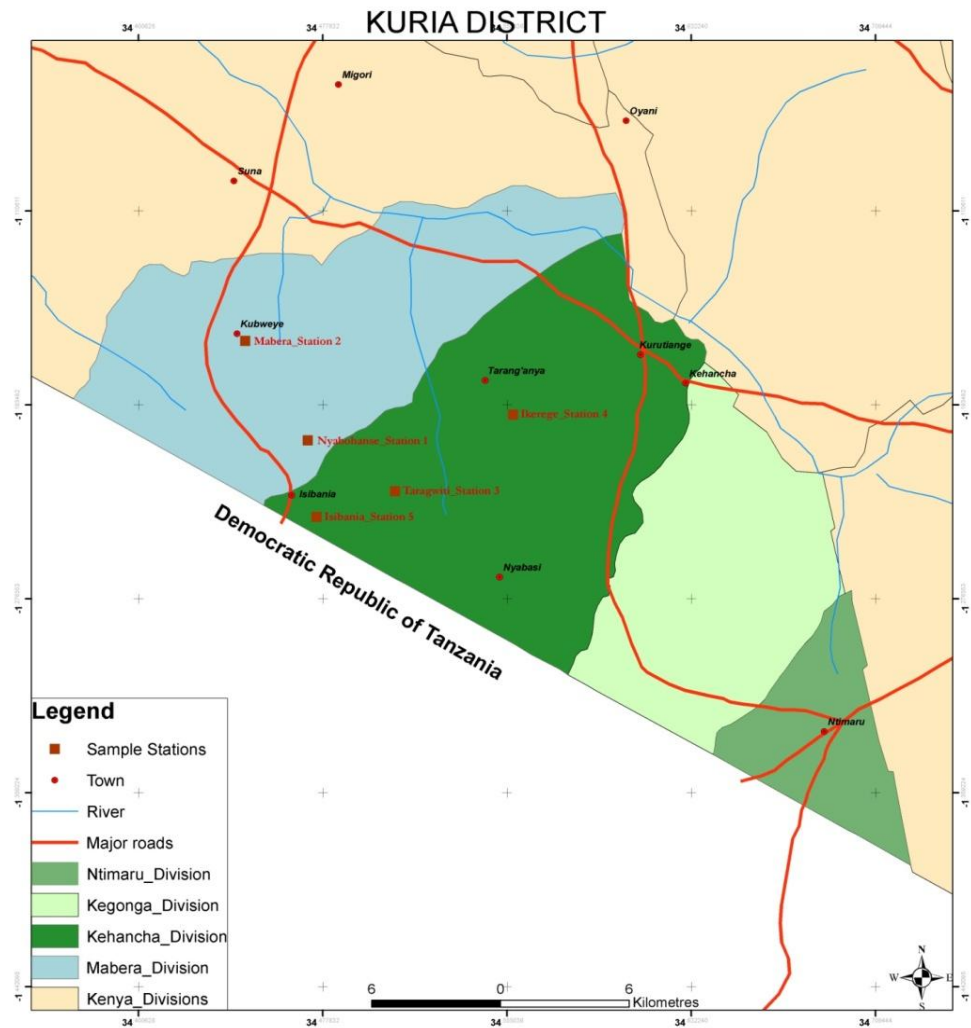


Figure 3.1: Map of Kuria district, showing divisions and sampling sites

3.2 Research design

The research design involved descriptive survey using a questionnaire to gather information from tobacco farmers and vegetable growers, and experimental that included, sampling of soils and vegetables in selected farms and markets, analysis of the levels of the pesticide residues and simulation study carried out using one of the vegetables (kales) to determine the rate of degradation of the pesticides.

3.3 Cleaning of glass apparatus

All glassware used in this study was soaked for 12 hours in freshly prepared chromic acid. They were then rinsed with distilled water and soaked in distilled water for about 6 hours to leach off any adsorbed chromic ions. Finally they were dried in an oven after rinsing and stored. The graduated glassware were not dried in the oven, but were put on racks and left to dry. Before use, the containers were rinsed with ethyl acetate.

3.4 Reagents and solvents

The reagents used were ethyl acetate, which is a good solvent for extraction (Kadenezki *et al.*, 1992; Fernandez-Alba *et al.*, 2002; Startin *et al.*, 2002), anhydrous sodium sulfate, methanol, methanoic acid and acetonitrile. All the reagents were of analar grade (pesticide residue grade), and were purchased from Kobian distributors limited (Nairobi, Kenya). The analytical standards for both methomyl 98% and acephate 97% were obtained from Hangzhou sino chemical co.ltd, China and Jiangsu fengshan group co.ltd, China respectively.

3.5 Sampling

3.5.1 Farmers' awareness of use and health effects of pesticides

Fieldwork study was done using oral interview and questionnaires to gather information on the peoples' awareness of the use and effects of pesticides. The questionnaires were first developed and pre tested using 40 farmers who were not part of the study, after which they were administered. The sampling for the awareness group was random.

3.5.2 Sampling of farms and vegetables

Selection of the farms for analysis was done using purposive sampling and snowballing. The farms selected were those with the vegetables of interest (kales, spinach and amaranth) where tobacco had been planted, and the two pesticides (methomyl and acephate) used. Random sampling was used to obtain the samples from the five stations as indicated in Figure 3.2 in (appendix IV). There were three farm stations and two market stations. From each farm station six samples in triplicates were collected while from the market stations three samples in triplicates were collected. The samples were collected once every month for five months during the dry season (Jan – Feb 2010) and wet season (march-may 2010). Station 2 did not have spinach between January - March, station 3 had no spinach in January and station 5 did not have spinach between February - April. Therefore a total of 147 samples were collected from the stations and analyzed.

3.5.3 Experimental garden

Soils where tobacco had been planted were collected and transported to Kenyatta University. Seedlings from the seedbeds used for tobacco were also collected and transplanted on the soils. These soils were homogenized, divided into three equal portions and placed 3 m apart from each other. On one portion the kales were sprayed with acephate, made by dissolving 1.25 g in 500 ml water the other with methomyl, made by dissolving 0.15 g in 500 ml water as required, while the controls were not sprayed. After growing for 4 weeks because kales can be harvested as soon as the leaves are large enough that is between 21-28 days (Rodriguez, 2010). The kales were sprayed and the samples collected from 30th to 43rd day after planting and 2nd to 15th day after spraying for analysis. In addition to the sprayed samples from the experimental garden, 14 samples in triplicates of the control were analyzed for the two pesticide residues where no further application of the pesticide was done. A total of 126 samples were collected from the experimental garden and analyzed.

3.6 Sample pretreatment

The samples collected for analysis were kept in plastic bags and frozen. Freezing was preferred to drying because some plants metabolize the pesticides very rapidly and some residues may no longer be present after wilting or drying (Manual of pesticide residue analysis, 2009). The types of vegetables analyzed in this study were, kales (*Brassica oleracea L.*), spinach (*Spinacia oleracea L.*) and amaranth (*Amaranthus retrolexus L.*).

3.7 Sample extraction

Prior to extraction the samples were rinsed with distilled water. A multiresidue method of extraction was used in this study. Multiresidue methods are very cost effective and are used in many laboratories. With regard to extraction efficiency, ethyl acetate has been shown to be equivalent to the water miscible solvents for both polar and non polar pesticides in vegetables, fruits and dry products. Pesticides are released and extracted efficiently (Hans *et al.*, 2007).

The vegetables were chopped and then blended to obtain a homogeneous sample. Using an analytical weighing balance (model Libror AEG 220), 10 g of the blended sample was weighed into a 250 ml flask for extraction to which 50 ml ethyl acetate and 25 g sodium sulfate were added and shaken for two hours at 80 cycles/min on a horizontal shaker. The ethyl acetate extract was filtered through a Whatman (No. 4) filter paper, and washed twice with portions of 25 ml each of ethyl acetate. The filtrate was collected in a 100 ml round bottomed flask and concentrated with a rotary evaporator to dryness. The extracts were reconstituted in 5 ml of methanol and stored in a refrigerator at 4°C. Before analysis, 1 ml of the extract was diluted with 4 ml distilled water and then filtered through a 0.45 µm syringe filter (Khan, 2005).

3.8 Preparation of stock solution and standards

In the preparation of the stock solution, 0.25 g of the pesticides (methomyl and acephate) standards was weighed into 250 ml volumetric flasks and dissolved to

the mark with methanol to make standard stock solution (1000 ppm). Six different concentrations of each pesticide that is, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm, for methomyl and 10 ppm, 20 ppm, 40 ppm 60 ppm 80 ppm and 100 ppm, were prepared from the stock solutions by serial dilutions. Standards were stored in refrigerator at 4 °C.

3.9 Method validation

The procedure and method was validated by use of matrix spikes and the reference sample (vegetables not sprayed). The reference sample was spiked with 50 ppm of the analytes and allowed to pass through the procedure as the sample. The percentage recovery was calculated using the formula given in Equation 3.1.

$$\% \text{ Recovery} = (\text{SSR} - \text{USR}) / \text{USR} \times 100 \dots \dots \dots (3.1)$$

Where, SSR – spiked sample result
USR – unspiked sample result

3.10 Sample analysis

The LC 10ADVP Shimadzu liquid chromatograph with SPD-10AVP Shimadzu UV-VIS detector and a Spherisob ODS 2, 5 micron (C18 250 ×3.2 mm s/no 40556) column manufactured in the year 2002 was used. The column conditions used were a flow rate of 0.4 ml/min, pressure of 116-118 Kgf, and oven temperature of 30 °C. Detection was at minimum area 100, stop time 15 min, ATT 2, 233 nm and 0.1000 UFS. The mobile phase used was 0.1% methanoic acid: acetonitrile (9:1), in isocratic elution.

In the analysis of samples, 20 µl portions of the sample and standard solutions were injected manually into the HPLC column and each sample was analyzed in triplicates. The concentration of the standard and peak area was used to draw a calibration curve that was used to determine the concentration of the residues. The amount of the methomyl and acephate pesticide residue was calculated using the relationship explained by Wang *et al.* (2008) and given in Equation 3.2.

$$C_{\text{res}} = (C_{\text{ref}} \times f_v) / W_{\text{sample}} \times 1/\text{rec} \dots \dots \dots (3.2)$$

Where,

C_{res} = concentration of residue in mg/kg

C_{ref} = concentration of analyte obtained from calibration curve (mg/ml).

$f_v = (v_1/v_2) \times (v_3/v_4)$

W_{sample} = weight of sample in g

rec = recovery

For this analysis,

$v_1 = 50$ ml

$v_2 = 100$ ml

$v_3 = 5$ ml

$v_4 = 1$ ml

$W_{\text{sample}} = 10$ g

rec = 92% for acephate and 95% for methomyl

3.11 Data analysis

The data was analyzed using statistical software for social scientists (SPSS version 11.5). T-test (independent) and ANOVA (one-way).

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Introduction

The results of the farmers' awareness of pesticide effects and analysis of pesticide residues in vegetables grown from Kuria West District are presented in this chapter. A total of 273 samples of the different types of vegetables were analyzed for methomyl and acephate. The vegetables included amaranth (*Amaranthus retroflexus L.*), kales (*Brassica oleracia L.*) and spinach (*Spinacia oleracia L.*). These vegetables were collected during dry season (January to February) and wet season (March to May). Triplicate samples of each vegetable were collected from each station and analyzed.

4.2 Method validation

The methods used in this study were validated as follows:

4.2.1 Questionnaires

The questionnaires were pre tested using farmers who were not part of the study. The farmers did not have any difficulty in answering the questionnaires.

4.2.2 Spiking and recovery

Spiking was done using 50 ppm and 100 ppm of the standards. This was done on daily basis before analysis of samples. The retention times for acephate and methomyl were at 4.648 min to 4.797 min and at 9.446 min to 9.783 min

respectively. Recoveries were done for 0.1 mg/kg, 0.5 mg/kg and 10 mg/kg for both methomyl and acephate. The values were acephate 92% and methomyl 95%. The detection limits for the pesticides were, Acephate (0.01 mg/kg) and Methomyl (0.0001 mg/kg).

4.3 Assessment of the farmers' awareness on the effects of acephate and methomyl pesticides and their use on vegetables.

This objective was achieved by use of questionnaires, which were first pretested in a pilot study carried out in July 2009. Farmers provided information on various issues that were considered when planting vegetables and the use of the two pesticides and their effects. Information obtained is shown by Figure 4.1 to 4.8.

4.3.1 Farmers' response on planting vegetables on the same soils used for tobacco

When farmers were interviewed about utilization of the soils used for tobacco in planting other crops like vegetables, their responses were as summarized in Figure 4.1.

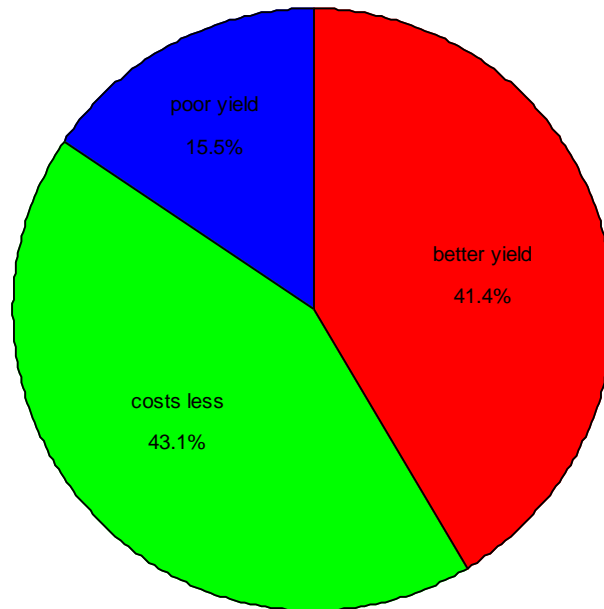


Figure 4.1: Percentages of response on rotating vegetables with tobacco

From Figure 4.1, 43.1% of the farmers interviewed reported that vegetables planted on the same soil used for tobacco costs less in terms of the input because the tobacco soils had fertilizer and pesticides, while 41.4% of the farmers were of the opinion that there was better yield because they were not attacked by pests easily due to the presence of the pesticides in the soils. However, 15.5% of the farmers argued that the pesticides used for tobacco were not fit for the vegetables therefore resulted in poor yield. These results showed evidence of rotation of tobacco with other crops including vegetables. A study by Ryan *et al.* (2010) revealed that vegetable crops and cotton are often rotated with soybeans and tobacco. Another study conducted by Hanscombe and Hanscombe (2008) also reported that vegetable crops were rotated between tobacco crops in order to avert a major food crisis that threatened lives and the high level of health enjoyed by the

average Cuban. Guy *et al.* (2002) also reported that in Quebec, rye and oats are often grown in rotation with vegetables and tobacco. It is therefore a common practice in many regions to rotate crops especially where land resources are limited.

4.3.2 vegetables planted on tobacco soil without further application of pesticides

Farmers were interviewed about their views on planting vegetables on soils used for tobacco without further applying the pesticides. Figure 4.2 shows the responses given by farmers on rotation of vegetables with tobacco without further spraying.

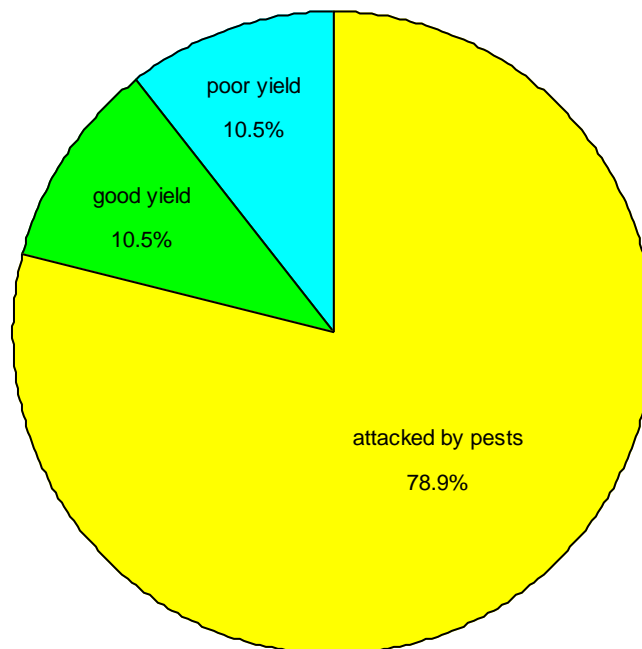


Figure 4.2: Farmers' response on rotation of vegetables with tobacco without further spraying

Figure 4.2 shows that, 78.9% of the farmers were of the opinion that when vegetables were grown on tobacco soil without further spraying they were attacked

by pests because most of the pests were common to both tobacco and vegetables. While 10.5% of the farmers reported that there was good yield because of the presence of the pesticides and fertilizer used on tobacco in the soils and 10.5% of the farmers said there was poor yield. This can be attributed to the fact that most of the pest, including mites, aphids and nematodes are common to both vegetables and tobacco hence if no further spraying was done the vegetables were bound to be attacked by pests at some time.

4.3.3 Application of pesticides used for tobacco on vegetables

Most of the farmers acquire pesticides for planting tobacco, after which the remaining pesticides are utilized on other crops. The farmers' responses on whether the pesticides used on tobacco were also used on vegetables are shown in Figure 4.3.

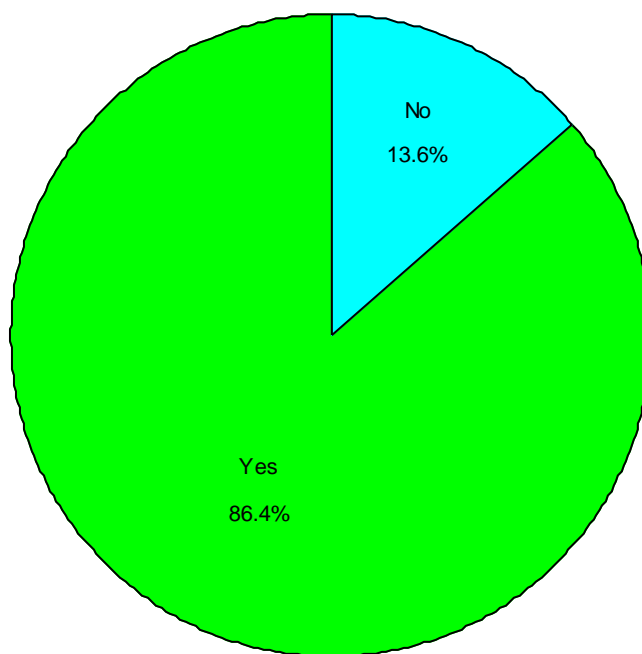


Figure 4.3: Farmers' responses on application of same pesticide used on tobacco on vegetables

From the results in Figure 4.3, 86.4% of the farmers admitted using the same pesticides for tobacco on the vegetables due to their availability and also lack of finances to buy the other pesticides like the pyrethroids, while 13.6% did not use acephate and methomyl on the vegetables but utilized the soils used for tobacco. Similar results have been reported by USGAO (2003) showing that most of the pesticides used on tobacco were also used on food crops. Pradyot (2007) also reported that as a result of the common pests attacking both vegetables and tobacco, carbamate pesticides were used on both the vegetables and tobacco.

4.3.4 Farmers' knowledge of the precautionary measures required when using pesticides

The responses of farmers on the knowledge of the precautionary measures required when using the pesticides are summarized in Figure 4.4.

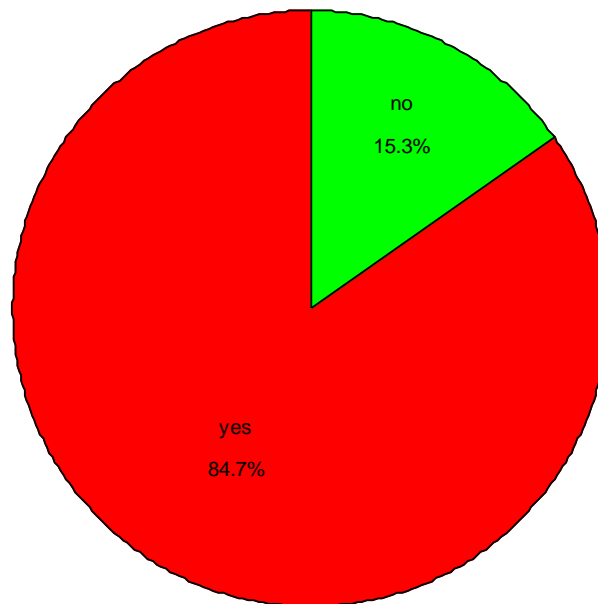


Figure 4.4: Farmers' response on knowledge of precautions

From Figure 4.4, it is clear that 84.7% of the farmers knew the precautions taken when applying the pesticides but this was more to do with the quantities applied at a given time and due to farming experience and social participation. This is supported by study carried out by Subashiny and Thiruchelvam (2008) revealing that farming experience and social participation contributed to farmers' knowledge on safe pesticide use. This study also found out that 15.3% did not take precautions when applying the pesticides. The literacy levels of farmers contributed a lot to the knowledge of precautions to be taken. A study by Jones *et*

al. (2009) reported that Mozambican farmers lack knowledge of the risks of pesticide use and do not take preventive measures. This could be the same reason for the 15.3% that did not know the precautions as outlined in this study. Another study by Yassin *et al.* (2002) also reported that farmers in Gaza strip were aware of the protective measures to be used when applying pesticides but did not take the precautions.

4.3.5 Sources of the instructions and precautions for use of pesticides

The farmers acquired the pesticides from the tobacco companies in the region, and when asked whether they received instructions, their responses are shown in Figure 4.5.

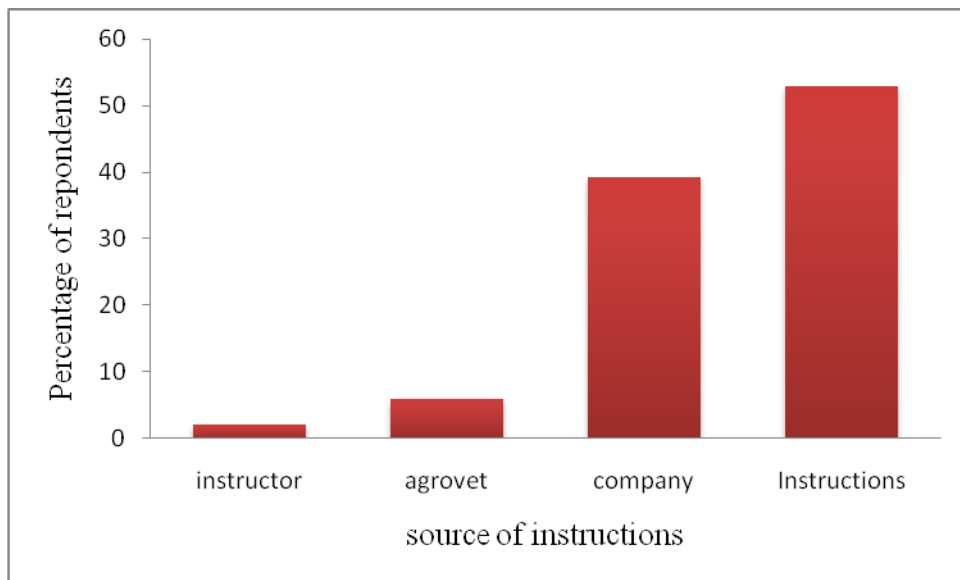


Figure 4.5: Responses of farmers on the sources of instructions and precautions

From Figure 4.5, 52.9% of the farmers read the instructions on their own, 39.2% received instructions from the tobacco companies that supplied them with the pesticides, 5.9% got instruction from the agrovet and 2.0% were helped by the instructors. From these results 47.1% did not understand the instructions on the pesticide labels on their own and therefore required assistance. It was therefore evident that there was lack of enough qualified personnel to provide the required information on instructions and the precautions to be taken. These findings were similar to a study by Lalah (2001) which revealed that due to lack of relevant data, equipment and qualified personnel, pesticides were applied following inappropriate recommendation. Subashiny and Thiruchelvam (2008), in their study of pesticide use in Pambaimadu Vavuniya District, recommended that increased social participation and effective extension programmes were important to improve farmers' knowledge on proper use of pesticides.

4.3.6 Farmers' awareness of the health effects of pesticides

When the farmers were asked whether they were aware of the side effects of these pesticides, their responses were as shown in Figure 4.6

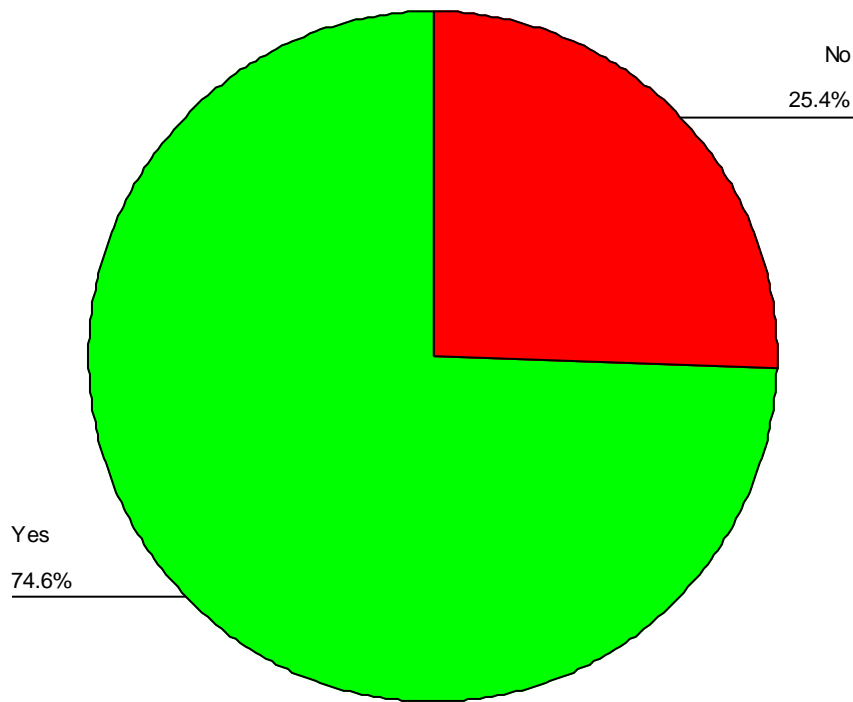


Figure 4.6: Farmers' responses on awareness of health effect of pesticides

As can be seen from Figure 4.6, 74.6 % of the farmers were aware of side effects of the pesticides, while 25.4% were not aware of the side effects. The percentage of those aware of the effects was significantly higher than that reported by Ouedraogo *et al.* (2010) that approximately 80% of pesticide applicators in Burkina Faso were illiterate hence ignorant about the potential dangers of pesticides to health and environment. The percentage of the farmers aware of the effects of pesticides in this study (74.6%) was probably attributed to farming experiences and social participation and not on literacy levels. Those not aware of the effects (25.4%) probably lacked experience and social participation. A study by Yassin *et al.* (2002) reported that 97.9% of the farm workers in Gaza strip reported high levels of knowledge on the health impacts of pesticides, and

moderate to high levels of knowledge were recorded on toxicity symptoms related to pesticides.

4.3.7 Farmers' response on use of protective clothing when applying pesticides

The farmers' response on use of protective clothing is shown in Figure 4.7.

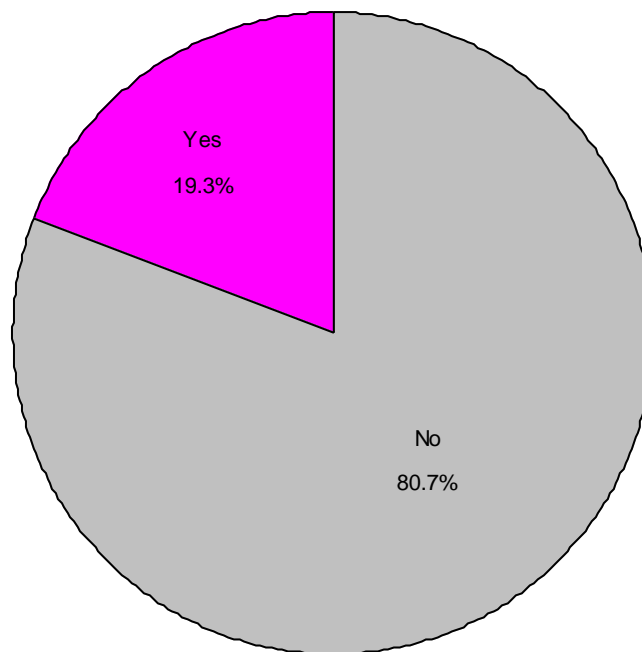


Figure 4.7: Farmers' responses on use of protective clothing when applying pesticides

From Figure 4.7, 80.7% of the farmers did not use any protective clothing during spraying, the reasons being they could not afford to buy them and also the companies did not offer them the protective clothing. This was confirmed by another study by Tchounwou *et al.* (2002) carried out in Egypt which reported that 98.9% of the farmers did not wear gloves, while 98.4% did not wear eye glasses

when mixing or applying pesticides and about 34% never wore long trousers and shoes when applying pesticides.

4.3.8 Farmers' response on health problems associated with application of pesticides

Pesticides have numerous health problems since tobacco farmers apply different pesticides without considering the protective aspect of the pesticides used. Farmers reported different health problems they suspected were brought about by the use of pesticides. These problems are shown in Figure 4.8.

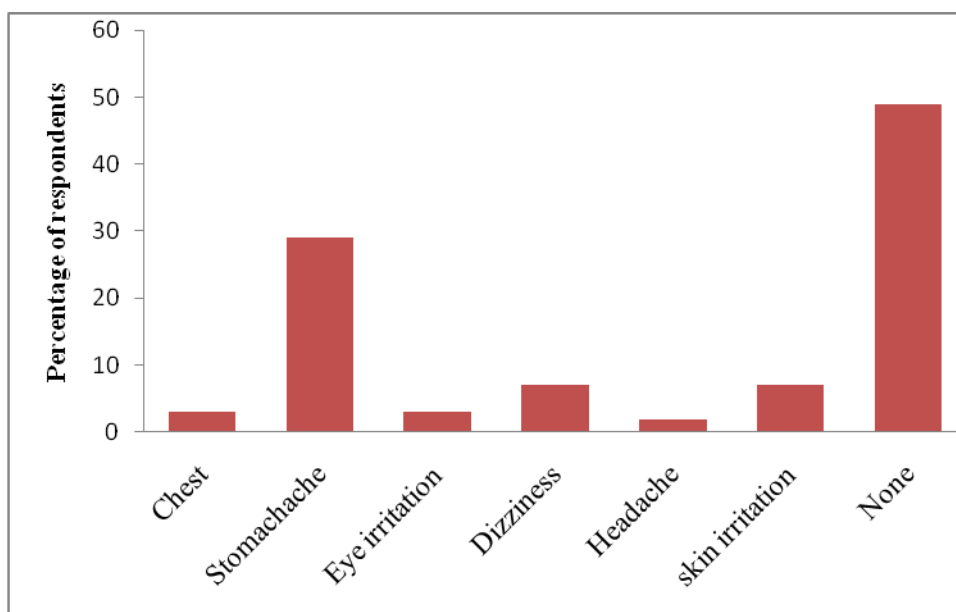


Figure 4.8: Farmers' response on health problems associated with application of the pesticides without protective clothing

Figure 4.8 shows that, there were different health problems perceived to be associated with the application of the pesticides. Among the farmers interviewed 49% could not associate the side effects with the application of the pesticides but

were of the opinion that those effects were symptoms of other complications apart from the application of the acephate and methomyl. The highest effect was stomachache with 28.8%, while the other effects had percentages below 10%. However it was hard to verify if these problems were caused by the pesticides without clinical studies. A similar study by Tchounwou *et al.* (2002) showed that 84% of the farmers in Egypt experienced discomfort during pesticide application. Among the clinical symptoms of pesticide poisoning reported were, nausea (53.2%), vomiting (13.8%), eye irritation (45.7%), tachycardia (12.2%), blurred vision (9.6%), abdominal cramps (15.4%), dizziness (33%), diarrhea (20.2%), head ache (59.6%), excessive salivation (5.3%), wheezing (16.5%), confusion (2.1%), sweating (14.9%) and coma (3.2%). According to Yassin *et al.* (2002), burning sensation in eyes/face was the commonest symptom (64.3%) with the prevalence of self-reported toxicity symptoms dependent on mixing and use of high concentration of pesticides.

4.4 Acephate residues levels in vegetables planted on same soils used for tobacco without further spraying and those sprayed

These were vegetables planted on the seedbeds that had been used for tobacco where there was no further spraying and those that were sprayed. Each sample was analyzed in triplicate. Table 4.1 shows the acephate residue levels for unsprayed and sprayed vegetables.

Table 4.1: Acephate mean residue (mg/Kg) levels for samples from farms.

Vegetable	Acephate residues in sprayed vegetables from farms used for tobacco (MEAN \pm SE)	Acephate residues in vegetables from seedbeds used for tobacco and not sprayed (MEAN \pm SE)	n	p
Amaranthus	-	0.1570 \pm 0.0520	33	-
Kales	0.0300 \pm 0.0050	0.1530 \pm 0.0430	66	0.012*
spinach	0.0270 \pm 0.0080	0.0430 \pm 0.0200	30	0.613
p- value	0.789	0.198		

*means are significantly different (at $\alpha=0.05$)

As can be seen from Table 4.1 and Appendix xxiii, the mean residue levels of acephate in the samples which were collected from the seedbeds had significantly higher levels of acephate residues as compared to the sprayed samples collected from the farms. This comparison was for only the kales and spinach since amaranth was not sprayed but was planted on the seedbeds. This may be due to the fact that the farmers used higher levels of pesticides before transplanting tobacco from the seedbeds, hence the significant difference in the means for the kales at 95% confidence level. Yen *et al.* (2000) reported that acephate had a longer half life in soil and could even lead to ground water contamination.

The residue levels in amaranth (0.1570 \pm 0.0520 mg/Kg) were significantly higher than the recommended minimum residue levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). These levels were also significantly higher than the reference dose (RfD) of 0.005 mg/Kg/day recommended by the US environmental protection agency (USEPA, 2002). This

could be due to the large quantities of pesticides applied on the seedbeds during the planting of tobacco which take a longer time to degrade.

The levels in kales were 0.0300 ± 0.0050 mg/Kg for sprayed and 0.1530 ± 0.0430 mg/Kg for unsprayed (seedbed) showing significant difference of the mean with 65 degrees of freedom. The t calculated was 2.736 which was more than the critical value of t which is 1.998 ($\alpha = 0.05$). The residue levels in the kales not sprayed (0.1530 ± 0.0430 mg/Kg) were significantly higher than the recommended levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). These levels were also significantly higher than the reference dose (RfD) of 0.005 mg/Kg/day recommended by the US environmental protection agency (USEPA, 2002). This shows that large quantities of the pesticides were applied on the seedbeds during the planting of tobacco, resulting in presence of high residues in the unsprayed kales which were planted on the seedbeds used for tobacco without further spraying. The levels of residues in sprayed kales (0.0300 ± 0.0050 mg/Kg) were within the recommended levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). They were however significantly higher than the reference dose (RfD) of 0.005 mg/Kg/day recommended by the US environmental protection agency (USEPA, 2002).

The residue levels in sprayed spinach (0.0270 ± 0.0080 mg/Kg) and those in unsprayed (seedbed) spinach (0.0430 ± 0.0200 mg/Kg), had no significant

difference in the means with 29 degrees of freedom, the calculated value of t was 0.382 which was less than the t critical which was 2.048 ($\alpha = 0.05$). Even though the means were not significantly different, the results show that people are still exposed to high levels of the residues. The residue levels in sprayed spinach (0.0270 ± 0.0080 mg/Kg) were within the recommended levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). They were however significantly higher than the reference dose (RfD) of 0.005 mg/kg/day recommended by the US environmental protection agency (USEPA, 2002). The residue levels in unsprayed spinach (0.0430 ± 0.0200 mg/Kg), were higher than the recommended levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). They were also significantly higher than the reference dose (RfD) of 0.005 mg/Kg/day recommended by the US environmental protection agency (USEPA, 2002). This could be as a result of farmers applying high quantities of acephate on the seedbeds used for tobacco. Table 4.2 shows the mean residue levels of the three vegetables from the market stations.

Table 4.2: Acephate mean (mg/Kg) residue levels of vegetables from market stations

Vegetable	Concentration (Mean \pm SE)	n	p
Amaranthus	0.0520 ± 0.0140^b	6	0.03
Kales	0.0270 ± 0.0050^{ab}	9	<0.005*
Spinach	0.0030 ± 0.0000^a	3	-
p-value	0.017		

NB: Mean values followed by the same small letter are not significantly different ($\alpha = 0.05$, SNK)*means are significantly different (at $\alpha=0.05$)

From the results in Table 4.2, amaranthus had significantly higher residue levels (0.0520 ± 0.0140 mg/Kg) than the recommended levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). The mean values for kales (0.0270 ± 0.0050 mg/Kg) were within the recommended levels (MRL = 0.0300 mg/Kg) by Canada's pesticide management regulatory agency (PMRA, 2008), pesticide regulation committee and the WHO (PRC, 2004). They were also significantly higher than the reference dose (RfD) of 0.005 mg/Kg/day recommended by the US environmental protection agency (USEPA, 2002).

Spinach is not a popular vegetable in the region therefore the samples from the market stations were very few. Most of the farmers plant spinach for their domestic use and there were few people who purchase directly from the farms. The acephate residue values (0.0030 ± 0.0000 mg/Kg) were very low and in other cases not detected. There was no significant difference between the means for spinach in the market samples. The post- hoc test showed that there was significant difference between the means ($p = 0.017$) of amaranthus (0.0520 ± 0.0140 mg/Kg) and spinach (0.0030 ± 0.0000 mg/Kg). These residue levels were within the recommended levels by the WHO (PRC, 2004) and Canada's pesticide management regulatory agency (PMRA, 2008). These results confirmed the presence of acephate residues in vegetables sold in the market. However acephate residue levels were lower than those obtained from the seedbeds. A study by Chang *et al.* (2005), also reported that among the vegetable samples analyzed in Taiwan in the year 1999-2004, 5 of the samples had acephate residues. Another

study by Liu *et al.* (2011), reported that the acephate residue data obtained from national food contamination monitoring program in china in the years 2001-2006, it was evident that exposures were higher than the acute reference dose (ACfD) in many cases with the exposure level of rural people being higher than the urban dwellers. Also vegetables contributed to acephate intake. A report by the National Pesticide Information Centre (NPIC, 2011) reported that out of 8315 samples of produce tested for acephate residues in the year 2006, 71 samples had detectable residues this including spinach, and watermelon.

4.5 Methomyl residues levels in vegetables planted on soils used for tobacco without further spraying and those sprayed

Table 4.3 shows the mean residue levels of methomyl in both the sprayed and those planted on the seedbed used for tobacco without further spraying (unsprayed). Only the kales and spinach levels were compared since amaranth was not sprayed.

Table 4.3: Methomyl mean residue(mg/Kg) levels in vegetables from farm stations

Vegetable	Methomyl residues in sprayed vegetables from farms used for tobacco (MEAN ± SE)	Methomyl residues in vegetables from seedbeds used for tobacco and not sprayed (MEAN± SE)	n	p
Amaranthus	-	0.0002 ± 0.0000	39	-
Kales	0.0010 ± 0.0003	0.0004 ± 0.0002	66	0.085
spinach	0.0003 ± 0.0002	0.0001 ± 0.0000	30	0.170
p- value	0.252	0.093		

NB: $\alpha = 0.05$

The results in Table 4.3 show that the mean residue levels for amaranthus were 0.0002 ± 0.0000 mg/Kg for unsprayed samples. Amaranthus vegetables are planted on the seedbeds that have been used for tobacco and are not sprayed. There were very low levels of methomyl residues in the amaranthus and spinach samples.

The mean residues levels in both sprayed and unsprayed kales were 0.0010 ± 0.0003 mg/Kg and 0.0004 ± 0.0002 mg/Kg respectively with 65 degrees of freedom, the calculated t value was 1.942 which was less than the critical t value 1.998 ($p=0.085$), hence there was no significant difference between the means. The mean residues levels in both sprayed and unsprayed spinach were 0.0003 ± 0.0002 mg/Kg and 0.0001 ± 0.0000 mg/Kg respectively with 29 degrees of freedom, t calculated being 1.476 and the t critical 2.048 ($p=0.170$) showing no significant difference between the means. These levels were significantly lower than the MRL (0.05 mg/Kg) recommended by FAO (2002), pesticide regulation committee and the WHO (PRC, 2004). These results are also shown in Appendix xxiv.

From Table 4.3, sprayed kales had the highest mean residues levels for methomyl (0.0010 ± 0.0003 mg/Kg) followed by unsprayed kales with a mean of 0.0004 ± 0.0002 mg/Kg. Unsprayed spinach had the lowest mean residue level (0.0001 ± 0.0000 mg/Kg) of methomyl. From these results it was clear that the farmers may have applied very little quantities of methomyl pesticide on the vegetables, or the presence could be due to the residues resulting from the use on tobacco since the

same soils are used. Table 4.4 shows results of methomyl residues in vegetables bought from the market.

Table 4.4: Methomyl mean (mg/Kg) residue levels from market stations

Vegetables	Concentration (MEAN \pm SE)	n	p
Amaranthus	0.0001 \pm 0.0001	6	0.003*
Kales	BLD	9	-
Spinach	0.0001 \pm 0.0000	3	-
p- value	0.703		

NB: * means significantly different (at $\alpha = 0.05$)

BLD- Below Detection Limit

It can be seen from Table 4.4 that the residue levels for methomyl were very low in all the three vegetables, or not within the detection limits of the HPLC for kales. This shows that very little methomyl is used or in some cases not used on the vegetables. These results are similar to a study carried out by Barkat (2005) which reported that out of the 608 samples of vegetables and fruits analyzed for pesticide residues in Pakistan, only 2.8% contained detectable levels of methomyl. Also a study by Chang *et al.* (2005) reported that among the vegetable samples analyzed for residues in Taiwan between the years 1999-2004, only 19 samples contained detectable methomyl residues.

4.6 Seasonal variation for sprayed vegetables

The vegetables were collected in a period of five months which reflected two seasons (dry and wet). Figure 4.9 shows the season variation for acephate residues in vegetables.

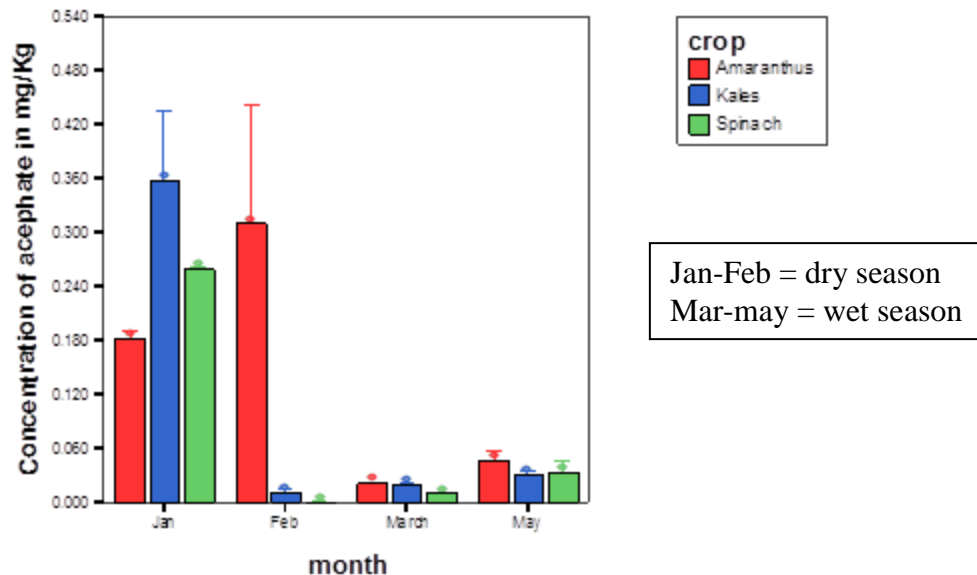


Figure 4.9: Season variation for acephate residue levels in vegetables

From Figure 4.9, the month of January had high mean acephate residue levels compared to the other months, with kales having the highest mean levels (0.3590 ± 0.0810 mg/Kg) followed by spinach (0.2600 ± 0.0050 mg/Kg) and then amaranthus (0.1800 ± 0.0100 mg/Kg). The high levels were significantly higher than the MRL of 0.03 mg/Kg recommended by pesticide regulation committee (PRC, 2004), and Canada’s pesticide management regulatory agency (PMRA, 2008). This could be due to the fact that the vegetables had been planted on the tobacco soils where large quantities of the pesticide had been used. Also January and February are dry seasons hence the vegetables may have been sprayed to prevent attack by pests which thrive during the dry season. The mean acephate

levels for the subsequent months were significantly lower than the MRL 0.03 mg/Kg.

The Figure 4.10 shows the season variation levels for methomyl residues.

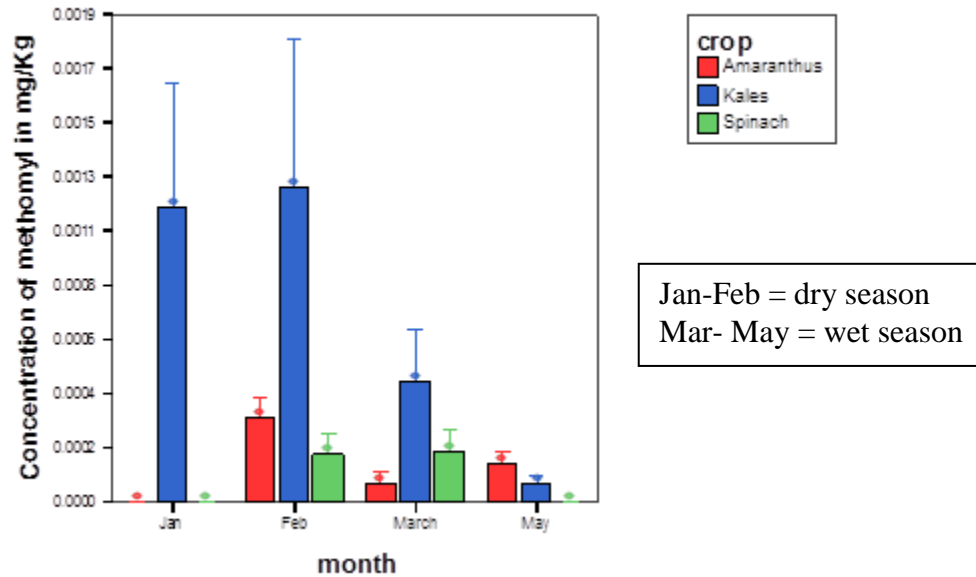


Figure 4.10: Season variation for methomyl residue levels in vegetables

From Figure 4.10, kales had the highest mean levels of methomyl residues in January, February and March but the levels were much lower than the MRL of 0.05 mg/Kg as recommended by FAO (2002), the pesticide regulation committee and the WHO (PRC, 2004). The methomyl residue levels in both the two seasons for the three vegetables were low. This may be due to the fact that very little quantities of methomyl are used on the vegetable or the residues could be as a result of the use of methomyl on tobacco which is retained by the soil for some

time. Table 4.5 shows the mean residue levels of acephate and methomyl for the two seasons.

Table 4.5: Mean residue levels of acephate and methomyl in dry and wet seasons (concentration in mg/Kg)

Pesticide	CONC. IN DRY SEASON \pm SE	CONC. IN WET SEASON \pm SE	N	P
Acephate	0.2058 \pm 0.0407	0.0235 \pm 0.0021	147	0.781
Methomyl	0.0007 \pm 0.0002	0.0002 \pm 0.0006	147	0.007*

*means are significantly different (at $\alpha = 0.05$)

From Table 4.5, it is clear that during the dry seasons the pesticide residues were higher than in the wet seasons. For acephate with 146 degrees of freedom, the t cal was 0.215 which was less than the t critical 1.976 ($p = 0.781$) hence no significant difference between the means. This is probably due to the fact that during the dry season the vegetables are affected more by pests and aphids than the wet seasons where the rains destroy their eggs and larvae therefore more spraying is done during the dry season. The low levels during the wet season could also be due to the wash off by rains.

The mean residues levels of acephate during the dry season (0.2058 ± 0.0407 mg/Kg) were slightly higher than the MRL of 0.03 mg/Kg recommended by Canada's pesticide management regulatory agency (PMRA, 2008), the pesticide regulation committee and the WHO (PRC, 2004). This was an indication that large quantities of acephate were applied on the seedbeds used for tobacco before transplantation during the dry season. The mean levels of acephate residue during the wet season of 0.0235 ± 0.0021 mg/Kg were significantly lower than the MRL

of 0.03 mg/Kg recommended by pesticide regulation committee and the WHO (PRC, 2004), and Canada's pesticide management regulatory agency (PMRA, 2008). This could be due to the fact that most farmers rarely apply pesticides on vegetables during wet seasons, because during the wet season the aphids and other insects do not affect vegetables much. The reproduction rate of these pests is very low during the wet season as most of their eggs and larvae are destroyed by the rains and weather. There is also a possibility that pesticides are washed off by the rain waters and that during the wet season the humidity tends to be high hence favoring the degradation of the pesticides.

For methomyl with 146 degrees of freedom the t_{cal} was 31.00 which was more than the $t_{critical}$ 1.976 ($p = 0.007$) hence the means were significantly different. The methomyl residue levels in both the two seasons of 0.0007 ± 0.0002 mg/Kg for dry and 0.0002 ± 0.0006 mg/Kg for wet were significantly lower than the MRL (0.05 mg/Kg) as recommended by FAO (2002), the pesticide regulation committee and the WHO (PRC, 2004). The reasons for these results are similar to those explained in the levels of acephate residue levels during the wet season. A study by Njagi (2007) reported that there were higher levels of the pyrethroid (deltamethrin and lambda-cyhalothrin) pesticide residues during the dry season compared to the wet season.

4.7 Levels of acephate and methomyl pesticide residues from the experimental garden

The reduction rate of the two pesticides and the results are shown in the Table 4.6. The seedlings were grown in tobacco soils and allowed to mature for 28 days after transplanting, before being sprayed. The samples were collected from the 2nd day after spraying continuously up to the 15th day, and each sample was analyzed in triplicate. The controls were also planted on soils from the seedbed used for tobacco, allowed to grow for 28 days and no spraying was done on these Kales. The control samples were also collected for 14 days consecutively and analyzed in triplicate. Table 4.6 shows the mean residue levels of acephate and methomyl from the experimental garden.

Table 4.6: Mean residue levels (mg/Kg) from the experimental garden using kales

Day after spraying/ planting	n	Acephate residues in sprayed kales (MEAN ± SE)	Methomyl residues in sprayed kales (MEAN ± SE)	Acephate residues in the controls (MEAN ± SE)	Methomyl residues in the controls (MEAN ± SE)
2/30	3	0.0370 ± 0.0003	0.0037±0.0001	0.0226±0.0001	BDL
3/31	3	0.0318 ± 0.0001	0.0060±0.0001	0.0201±0.0000	BDL
4/32	3	0.0309 ± 0.0002	0.0055±0.0001	0.0182±0.0001	BDL
5/33	3	0.0306 ± 0.0002	0.0044±0.0002	0.0163±0.0001	BDL
6/34	3	0.0266 ± 0.0002	0.0033±0.0001	0.0153±0.0001	BDL
7/35	3	0.0259 ± 0.0002	0.0032±0.0001	0.0148±0.0001	BDL
8/36	3	0.0259 ± 0.0001	0.0031±0.0001	0.0134±0.0001	BDL
9/37	3	0.0252 ± 0.0001	0.0029±0.0002	0.0127±0.0001	BDL
10/38	3	0.0249 ± 0.0002	0.0026±0.0001	0.0124±0.0001	BDL
11/39	3	0.0228 ± 0.0001	0.0026±0.0001	0.0117±0.0001	BDL
12/40	3	0.0212 ± 0.0001	0.0025±0.0001	0.0107±0.0001	BDL
13/41	3	0.0207 ± 0.0002	0.0024±0.0002	BDL	BDL
14/42	3	0.0180 ± 0.0001	0.0004±0.0000	BDL	BDL
15/43	3	0.0115 ± 0.0001	0.0003±0.0001	BDL	BDL

BDL-Below Detection Limit

From Table 4.6, kales had acephate and methomyl residues even 15 days after spraying. The control samples contained acephate residues even after 40 days after planting while no methomyl was detected 30 days after planting when the analysis started. The presence of the acephate residues in the controls may be as a result of

large quantities of acephate used on the tobacco seedbeds and hence were absorbed by the vegetables from the soil. This is similar to a report by National pesticide Information Centre (NPIC, 2011) which states that acephate is absorbed by plants quickly from the soil with its half-life ranging between 4.5-32 days.

When the results from the experimental garden are compared with those obtained in this study, it is clear that there is a possibility that the sprayed kales were harvested after 5 days because the acephate mean residue levels for the sprayed kales from the study was 0.0300 ± 0.0050 mg/Kg while that from the experimental garden by the 5th day was 0.0306 ± 0.0002 mg/Kg. These levels were significantly lower than those reported by FAO (2003) in Spain where harvesting should be done after 15 days with three trials in some *Brassica* vegetables giving acephate residues as follows; 0.05, 0.08 and 0.18 mg/Kg. The acephate residue level by the 15th day was 0.0115 ± 0.0001 mg/Kg which was significantly lower than the MRL of 0.03 mg/Kg recommended by pesticide regulation committee and the WHO (PRC, 2004) and Canada's pesticide management regulatory agency (PMRA, 2008). There were acephate residues in the controls although the levels were significantly lower than the recommended MRL of 0.03 mg/Kg. This could be due to the longer half-life of acephate which ranges between 4.5-32 days as reported by National pesticide Information Centre (NPIC, 2011).

The methomyl residues from the sprayed kales in the study were significantly lower than the MRL of 0.05 mg/kg. By the 15th day the methomyl residue level was 0.0003 ± 0.0001 mg/Kg very much lower than the MRL of 0.05 mg/Kg

recommended by FAO (2002), and pesticide regulation committee and the WHO (PRC, 2004). The levels found in this study by the 7th day were 0.0032 ± 0.0001 mg/Kg which were significantly lower than those found in a study by Mohamed and Saad (1995) which reported the rate of disappearance of foliage applied methomyl from some vegetables. The residue levels were 0.55, 0.2 and 0.6 mg/Kg seven days after methomyl had been applied to strawberries, tomatoes and cucumbers respectively (Mohamed and Saad, 1995). Mohamed *et al.* (1987) reported that after application of methomyl 90% under field conditions to follow up decline of residues in tomato and cabbage plants indicated that levels of methomyl residues were found to be below tolerance levels after the 3rd day from application.

Comparing the levels of methomyl in the experimental garden with those from the sprayed kales in the study it can be seen that those from the study were significantly lower than those from the experimental garden. The methomyl residues were not detected in the controls, indicating that very low levels of methomyl were used or possibly not used on the seedbeds during planting of tobacco. The other reason is due to the low persistency of methomyl in soil, no residue of methomyl is expected in crops planted where it has been used (EFSA, 2008).

From the results, it is clear that methomyl reduced at a higher rate than acephate where by the 15th day the methomyl residue level was 0.0003 ± 0.0001 mg/Kg very much lower than the MRL (0.05 mg/Kg) recommended by FAO (2002), and

pesticide regulation committee and the WHO (PRC, 2004). While the acephate residue level by the 15th day was 0.0115 ± 0.0001 mg/Kg which was significantly lower than the MRL (0.03 mg/Kg) recommended by pesticide regulation committee and the WHO (PRC, 2004) and Canada's pesticide management regulatory agency (PMRA, 2008).

A plot of the log of the concentrations of acephate and methomyl residues for the kales sprayed with the pesticides, against the day are shown in Figures 4.11 and 4.12 for acephate and methomyl respectively.

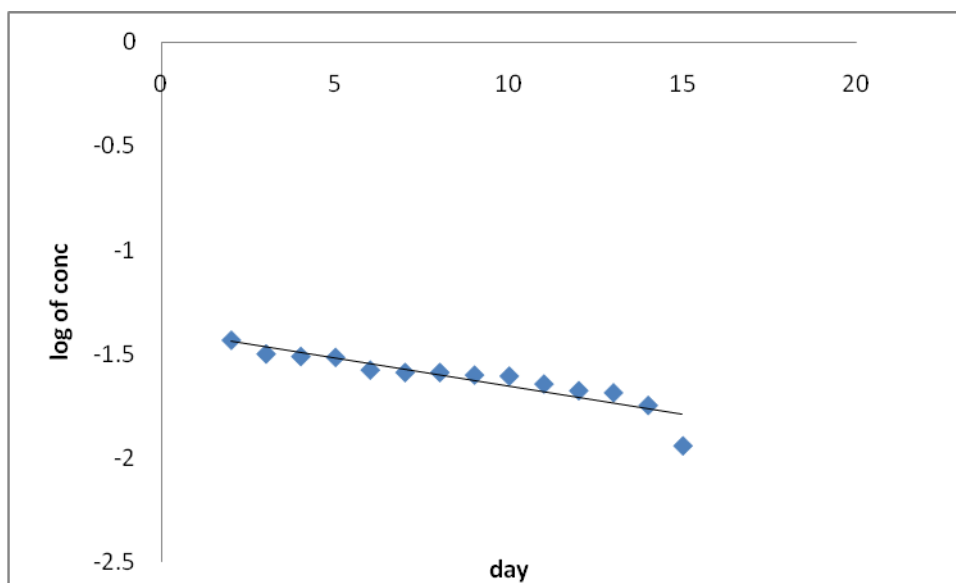


Figure 4.11: Degradation of acephate

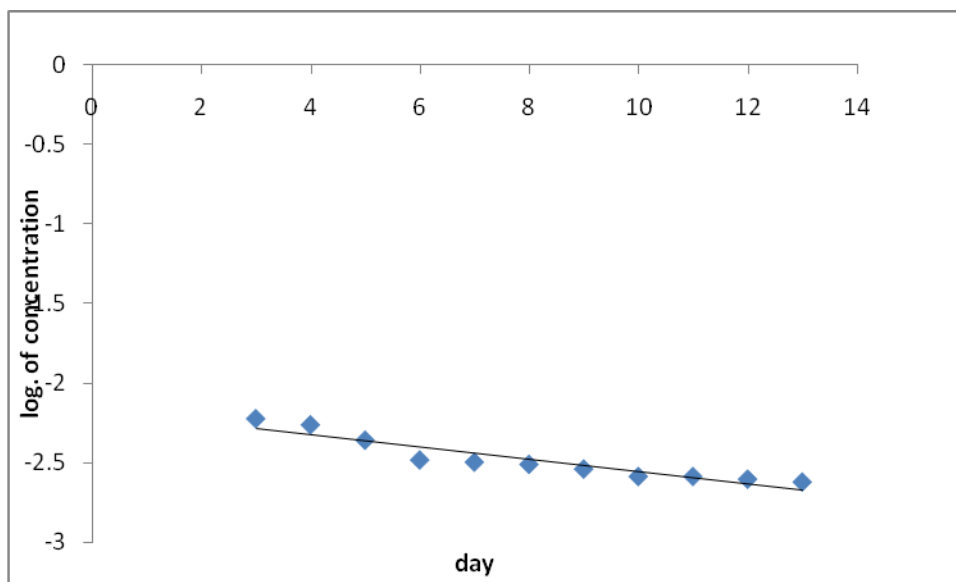


Fig. 4.12: Degradation of methomyl

From Figures 4.11 and 4.12 it can be seen that the reduction rate of the pesticides followed first order kinetics. By the 15th day when the vegetables were harvested residue levels were within the MRLs of 0.03 mg/Kg acephate and 0.05 mg/Kg methomyl recommended by the pesticide regulation committee and the WHO (PRC, 2004). Acephate had a lower level of reduction as compared to methomyl this may have been due to the physiochemical property of these pesticides. The lower degree of reduction of acephate may have resulted to lesser extent of disappearance. These results were similar to a study carried out by Chai *et al.* (2009) which reported that the dissipation of acephate followed first order kinetics with vegetable shading retarding the pesticide degradation in soil. Another study by Benejee *et al.* (2006) also reported that the dissipation of methomyl followed first order kinetics at standard and double-dose application.

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

From the present study, the following conclusions can be made;

- i. From the results, 74.6% of the farmers interviewed, were aware of the side effects of these pesticides, while 86.4% of Farmers interviewed applied the same pesticides on both tobacco and vegetables,
- ii. Most farmers (84.7%) interviewed were aware of the precautions taken in the application of pesticides but 81.4% of the farmers interviewed did not use any protective clothing during application of the pesticides.
- iii. The vegetables planted on the same soils used for tobacco growing were found to contain pesticide residues, with those from the seedbeds (with no further spraying) containing significantly higher levels during the dry season. The residue levels of acephate were significantly higher than those of methomyl during both the seasons.
- iv. The mean residue levels for acephate were significantly higher during the dry season than during the wet season. Most of the levels exceeded the MRL of 0.03 mg/kg recommended by pesticide regulation committee, the WHO (PRC, 2004) and Canada's pesticide management regulatory agency (PMRA, 2008). On the other hand the residue levels for methomyl were

low in all the seasons with all of them below the MRL of 0.05mg/kg by the same regulatory bodies. Generally there were lower levels of the pesticide residues during the wet seasons than the dry season.

- v. The reduction rate study carried out found that methomyl had a higher degradation rate than acephate which may explain why the residues of methomyl were very low and sometimes not detected.

5.3 Recommendations

5.3.1 Recommendation from the study

- i. Although the farmers were aware of the side effects of the pesticides, they did not use protective clothing during the use of the pesticides. The farmers should be educated on the necessity of using protective clothing during the application of the pesticides. The farmers should also be sensitized on the long term effects of the pesticides.
- ii. The farmers should be sensitized on the use of other pesticides on vegetables which are safer than acephate and methomyl, and those that degrade faster like pyrethroids.
- iii. If the farmers have to use acephate and methomyl pesticides on vegetables, then the correct quantities should be applied and harvesting done after the 15 day recommended.

- iv. Tobacco being a pesticide dependent crop, it is not advisable to intercrop it with vegetables due to the high residue levels found in the vegetables which are a risk to the consumers because other vegetables are eaten when row. If the tobacco soils are used then there should be no further application of acephate and methomyl pesticides.

5.3.2 Recommendation for further research

This study recommends the following,

- a. Research should be carried out to determine other pesticides in the vegetables considered in this study,
- b. Research should be carried out on the analysis of acephate, methomyl and other pesticides in:
 - i. Other vegetables not considered in this study, grains and fruits.
 - ii. Other matrices like soil, water and air.
 - iii. Green house produce, soils and air.
- c. The degradation products of these pesticides and their effects on organisms.

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APPENDICIES

APPENDIX I

INTERVIEW SCHEDULE FOR FARMERS

N.B: The information provided in this questionnaire will be used for academic purpose only.

1. Which month do you plant Tobacco?
2. Which pesticides do you apply on Tobacco?
3. Where do you get the pesticides from?
4. Do you wear protective clothing when applying pesticides?
5. Are you aware of any effects of the pesticides?
6. Have you ever had any health problem associated with the application of the pesticides? If yes explain.
7. Do you plant other food crops on the same pieces of land used for Tobacco? If yes specify
8. Do you apply the same pesticides used on Tobacco on vegetables?
9. How soon do you harvest the vegetables, after application of pesticide?
10. (a). Do you receive any information regarding the precautions to be taken when (b). If yes by who?
11. What is the difference between the vegetables planted on a different piece of land not used for tobacco and those planted together with tobacco?
12. What happens to the vegetables planted on the piece of land used for tobacco without any additional pesticides being used?

Thank you.

APPENDIX II

Seed bed where both vegetable and tobacco are.



APPENDIX III

Garden of vegetables utilizing the seedbeds used for tobacco



APPENDIX IV

APPENDIX V

Concentration of analyte



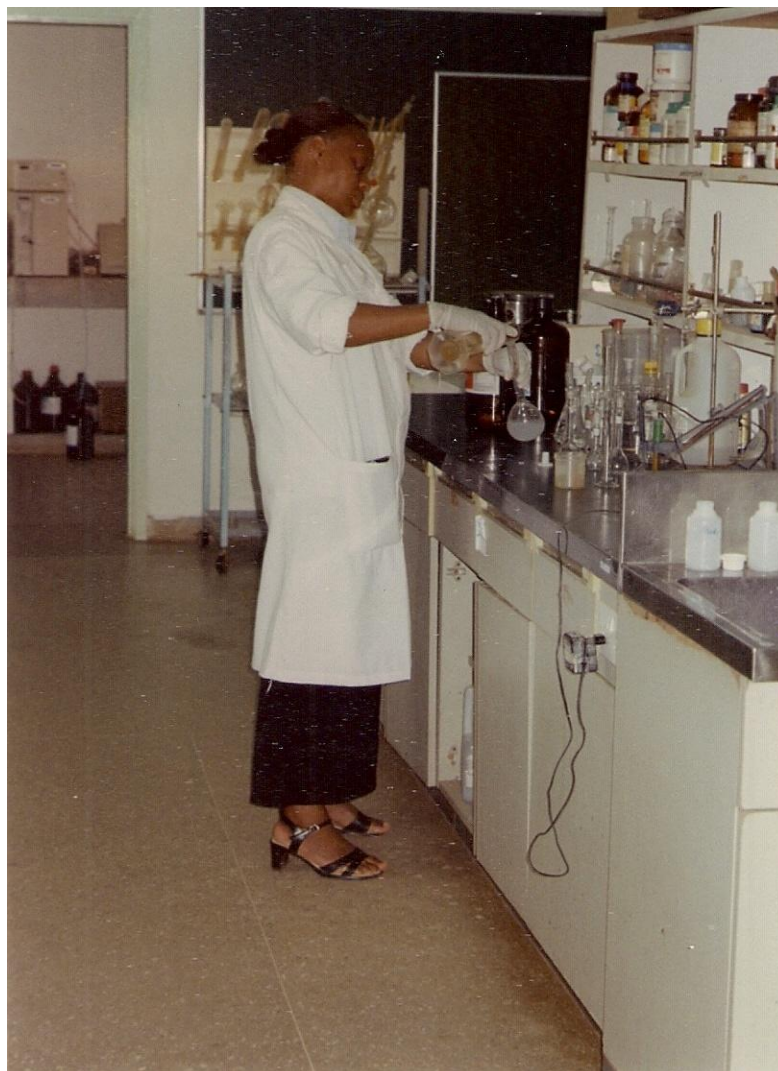
APPENDIX VI

Filtration of sample using a syringe filter.



APPENDIX VII

Preparation of pesticides standards.



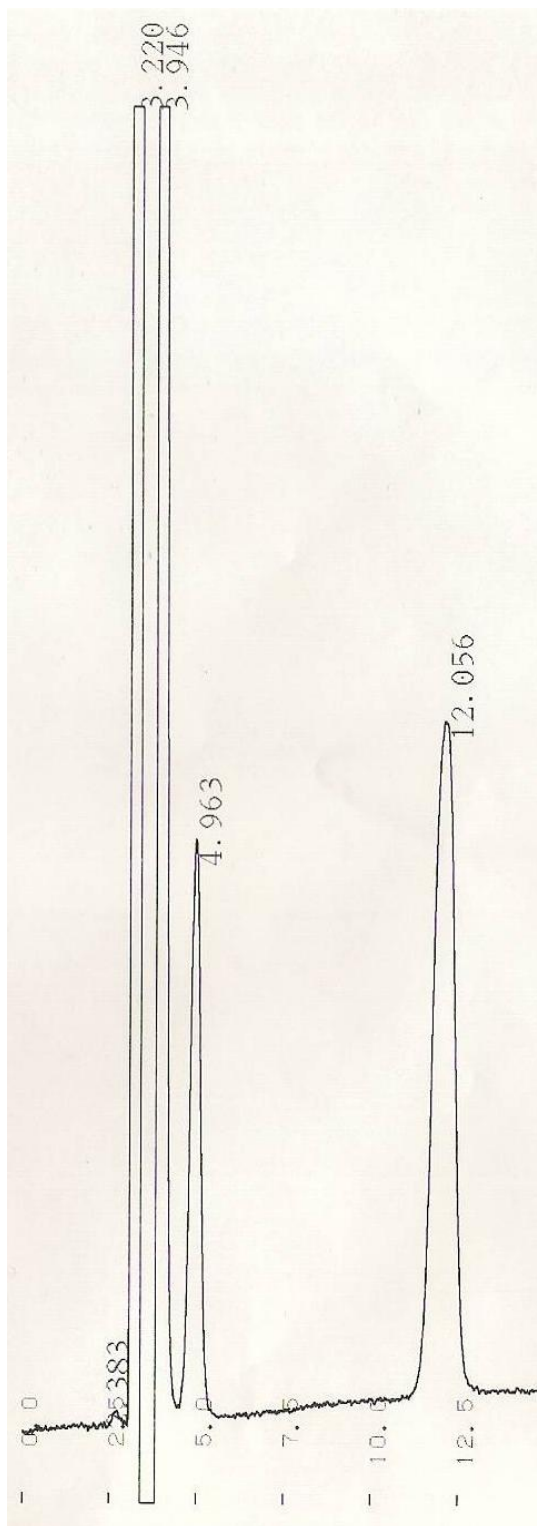
APPENDIX VIII

Injection of sample into the HPLC column.



APPENDIX IX

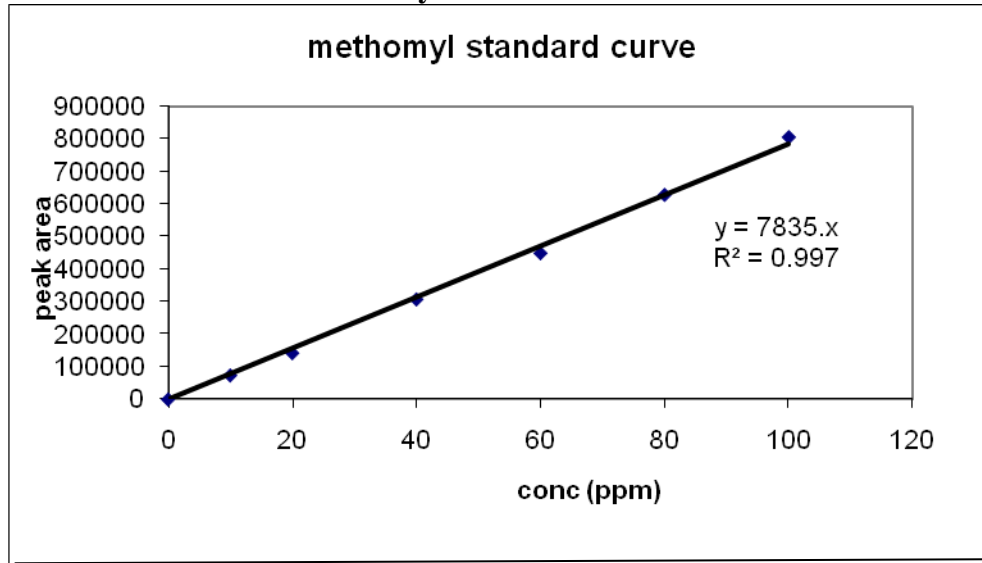
Chromatogram for acephate and methomyl obtained using HPLC



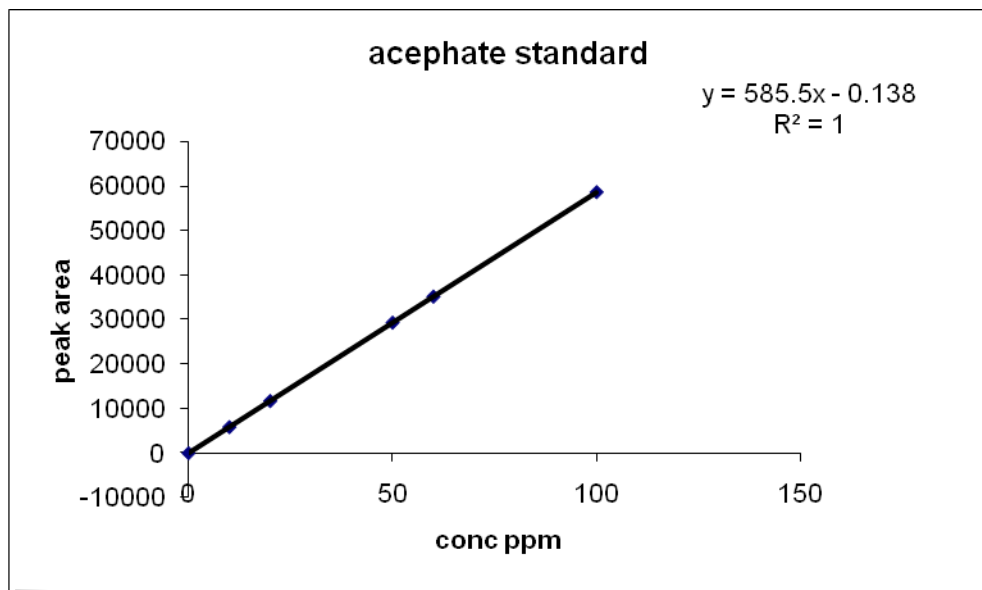
4.963-Acephate
12.056-Methomyl

APPENDIX X

Calibration curve for methomyl

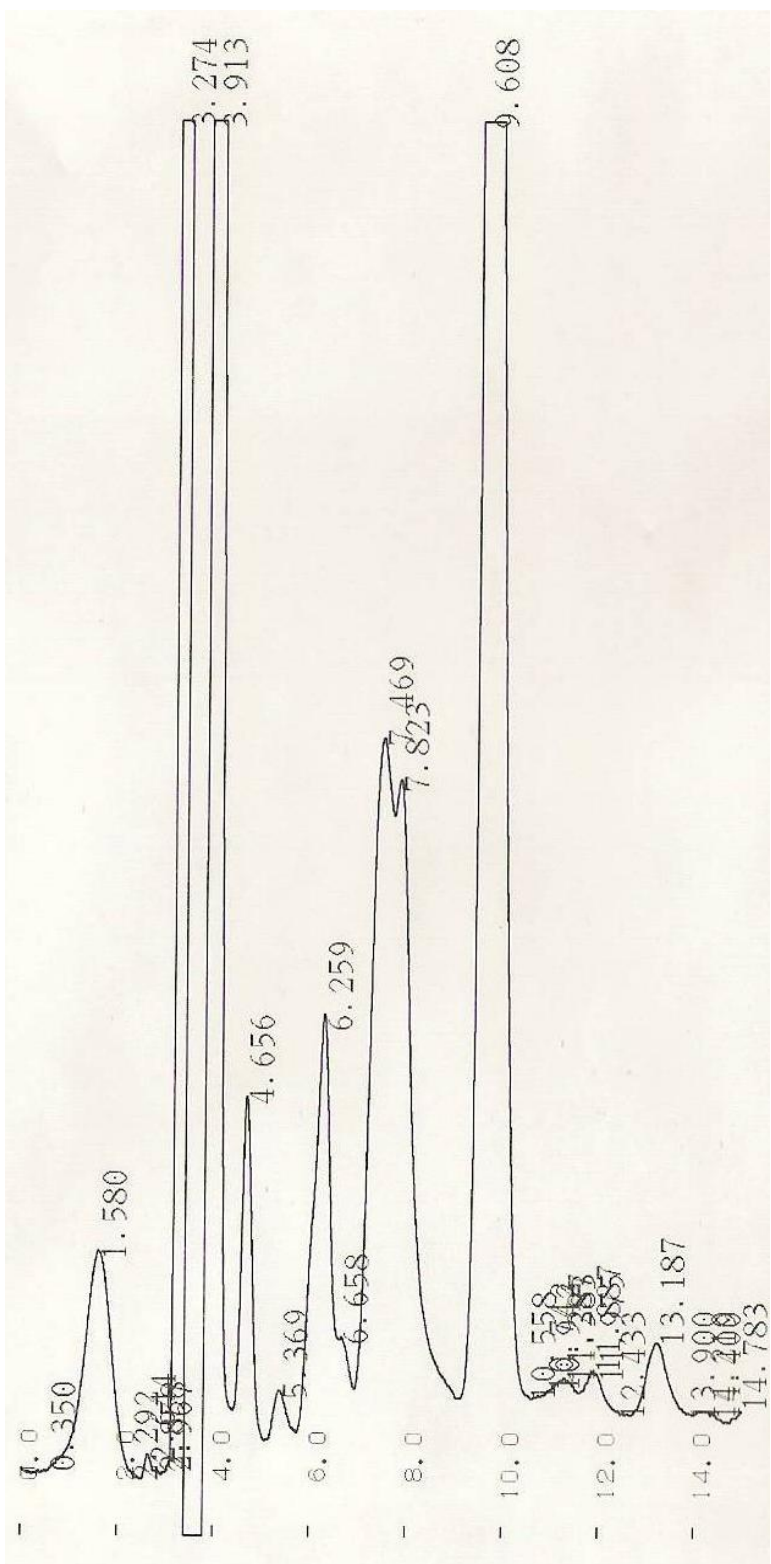


Calibration curve for acephate



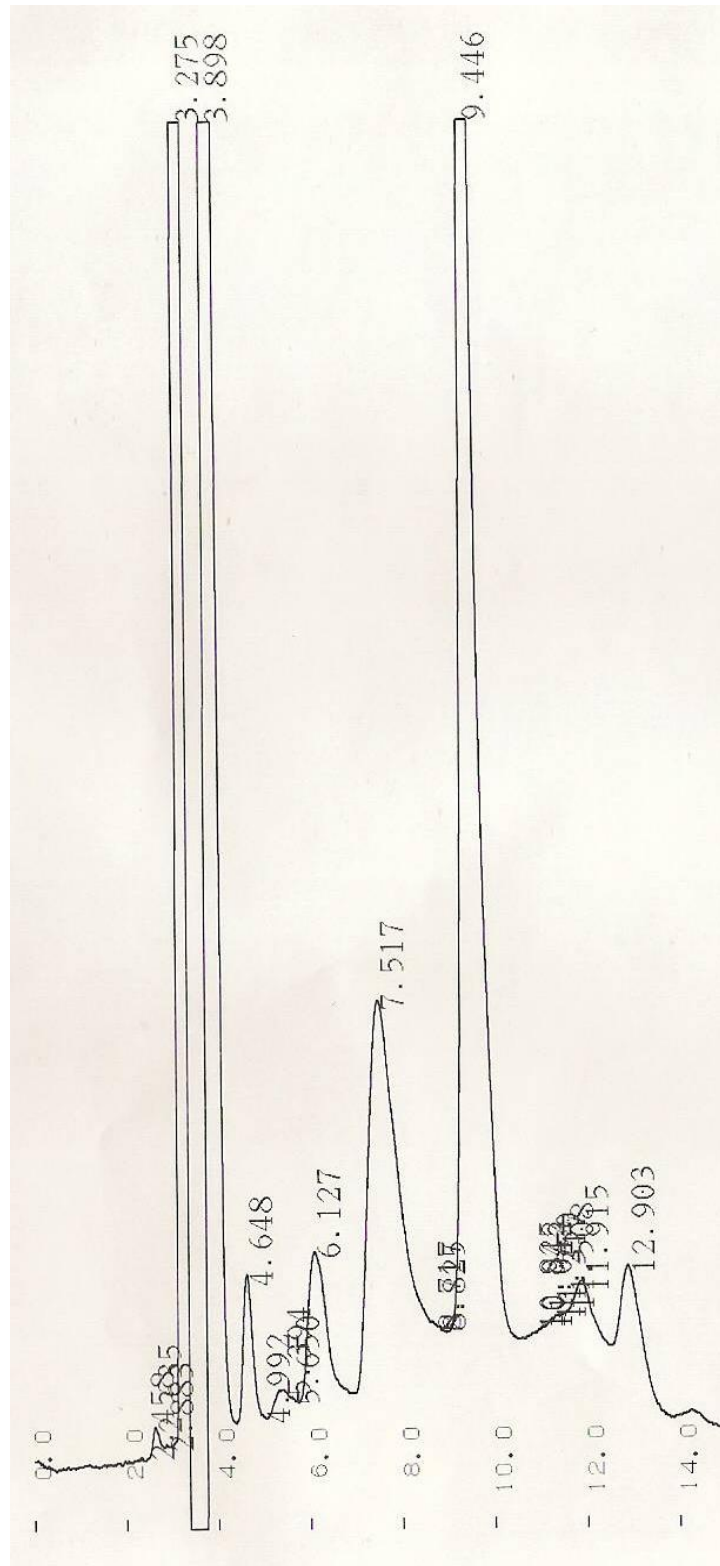
APPENDIX XI

Chromatogram for amaranthus blank spiked with pesticide mix



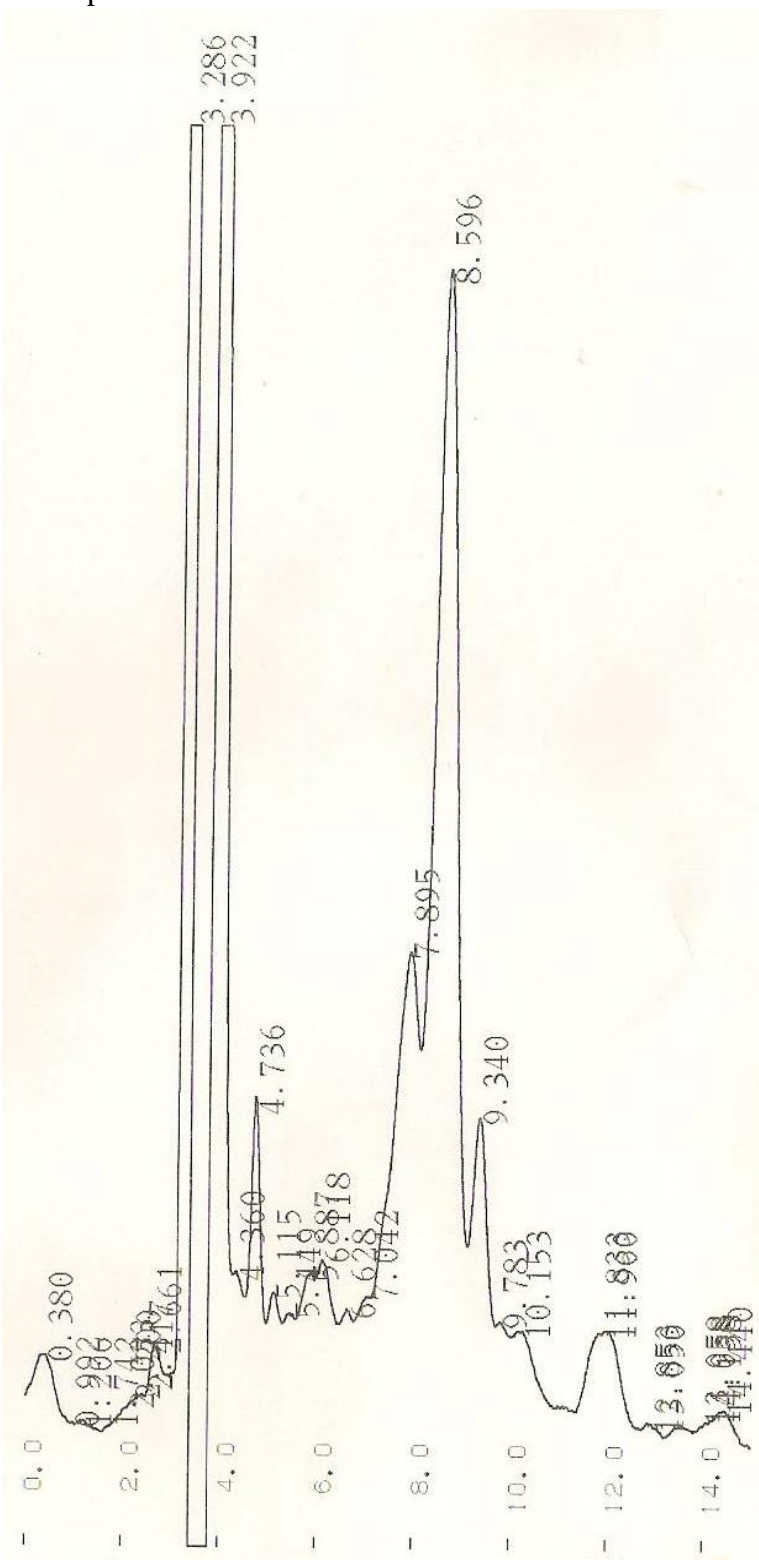
APPENDIX XII

Chromatogram for spinach blank spiked pesticide mix



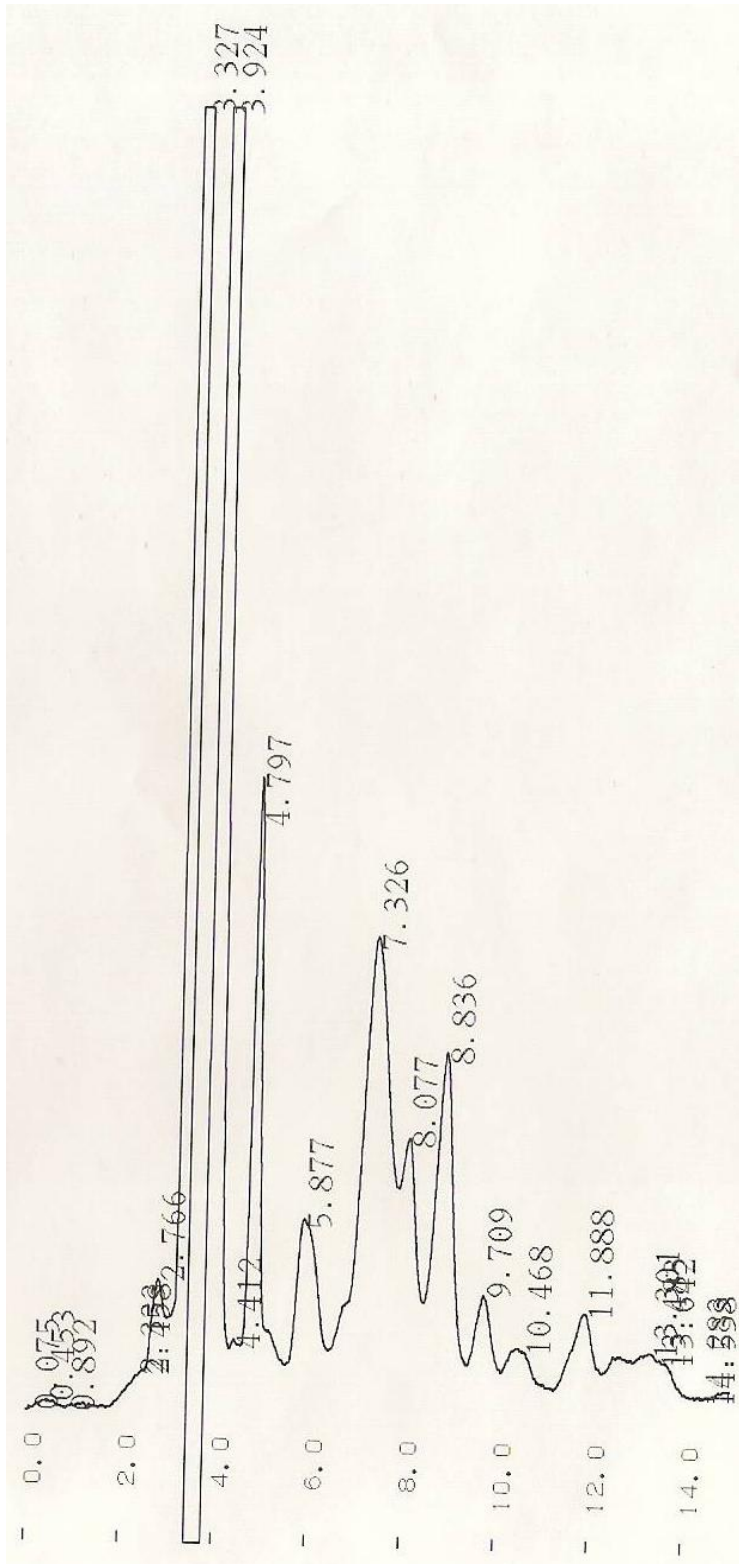
APPENDIX XIII

Chromatogram for a sample of kales from isibania market.



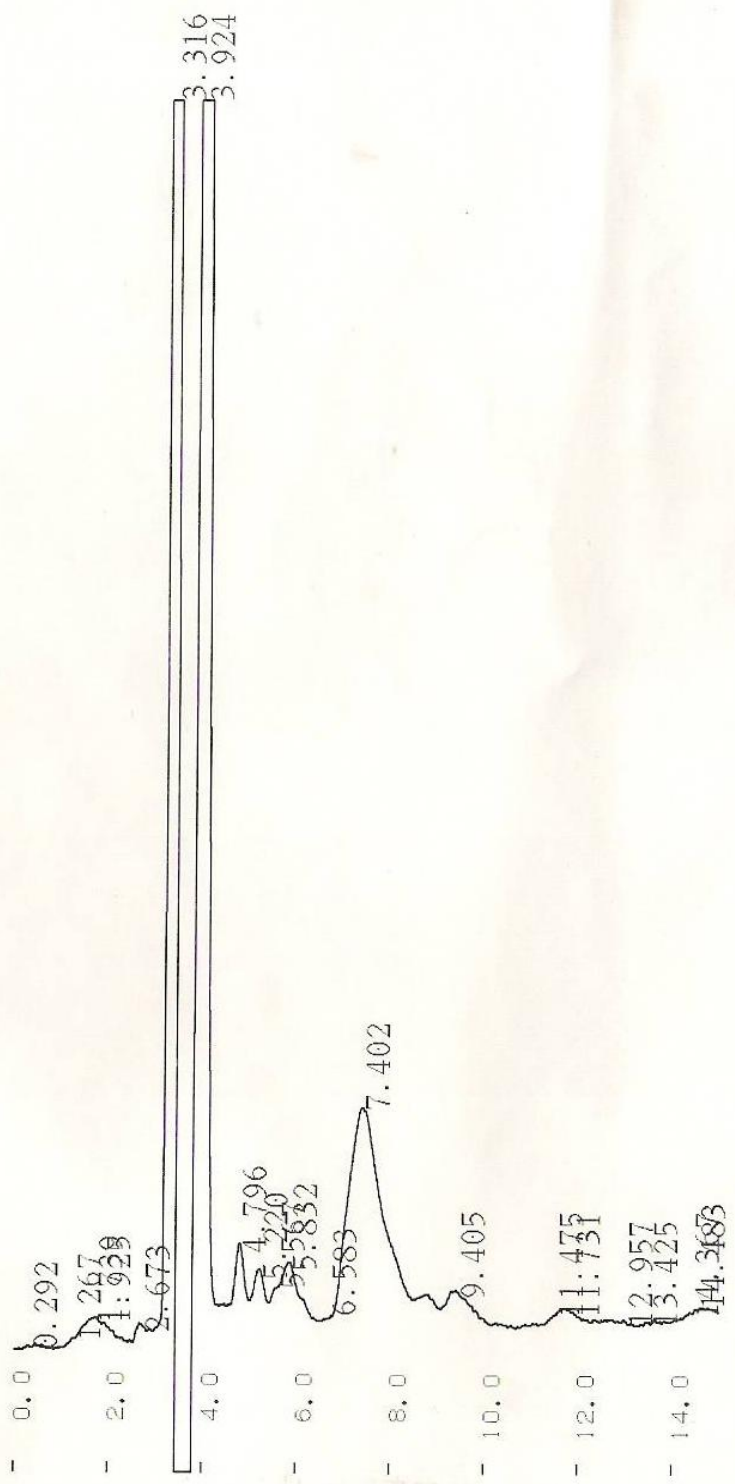
APPENDIX XIV

Chromatogram for a sample of kales sprayed station 3.



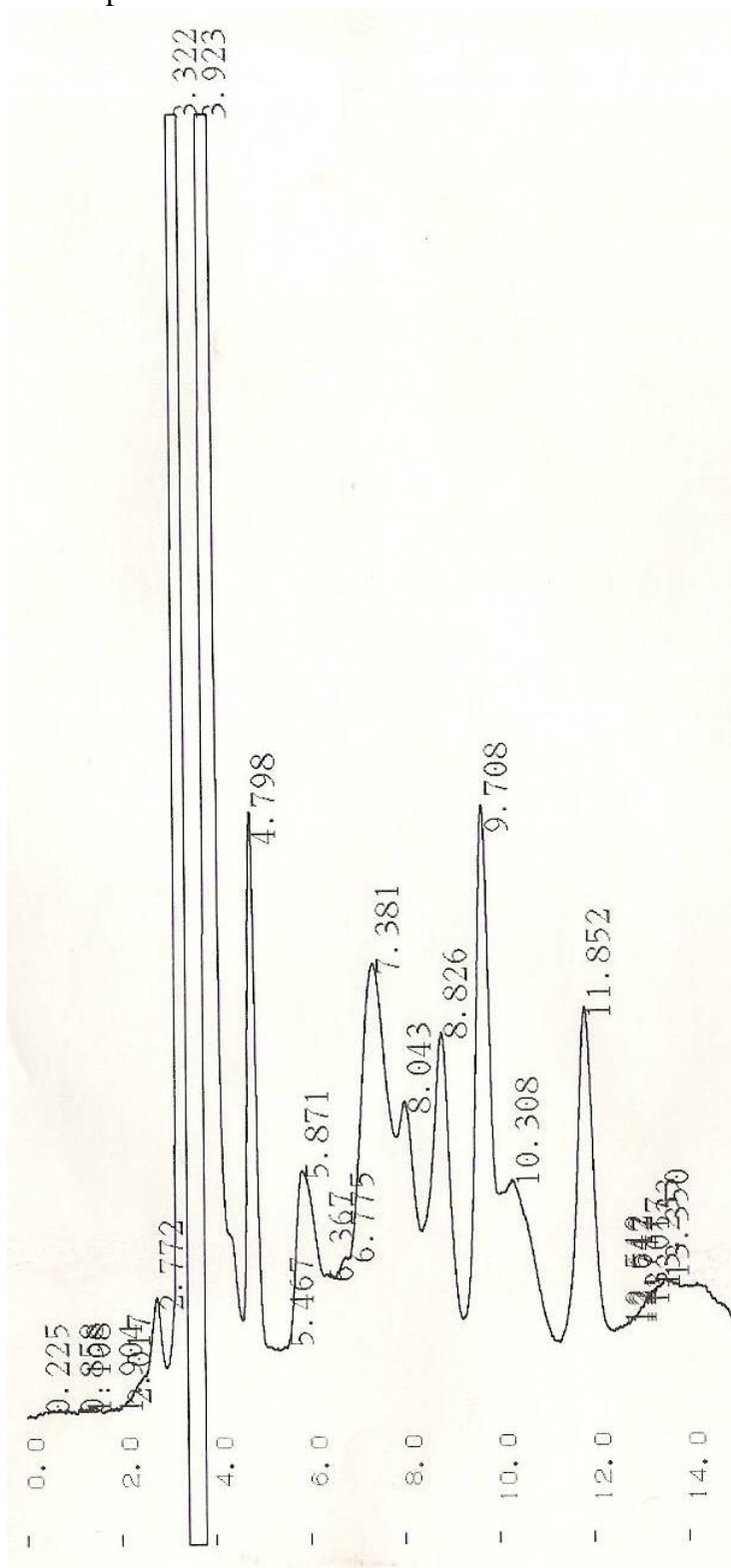
APPENDIX XV

Chromatogram for a sample of amaranthus from isibania market.



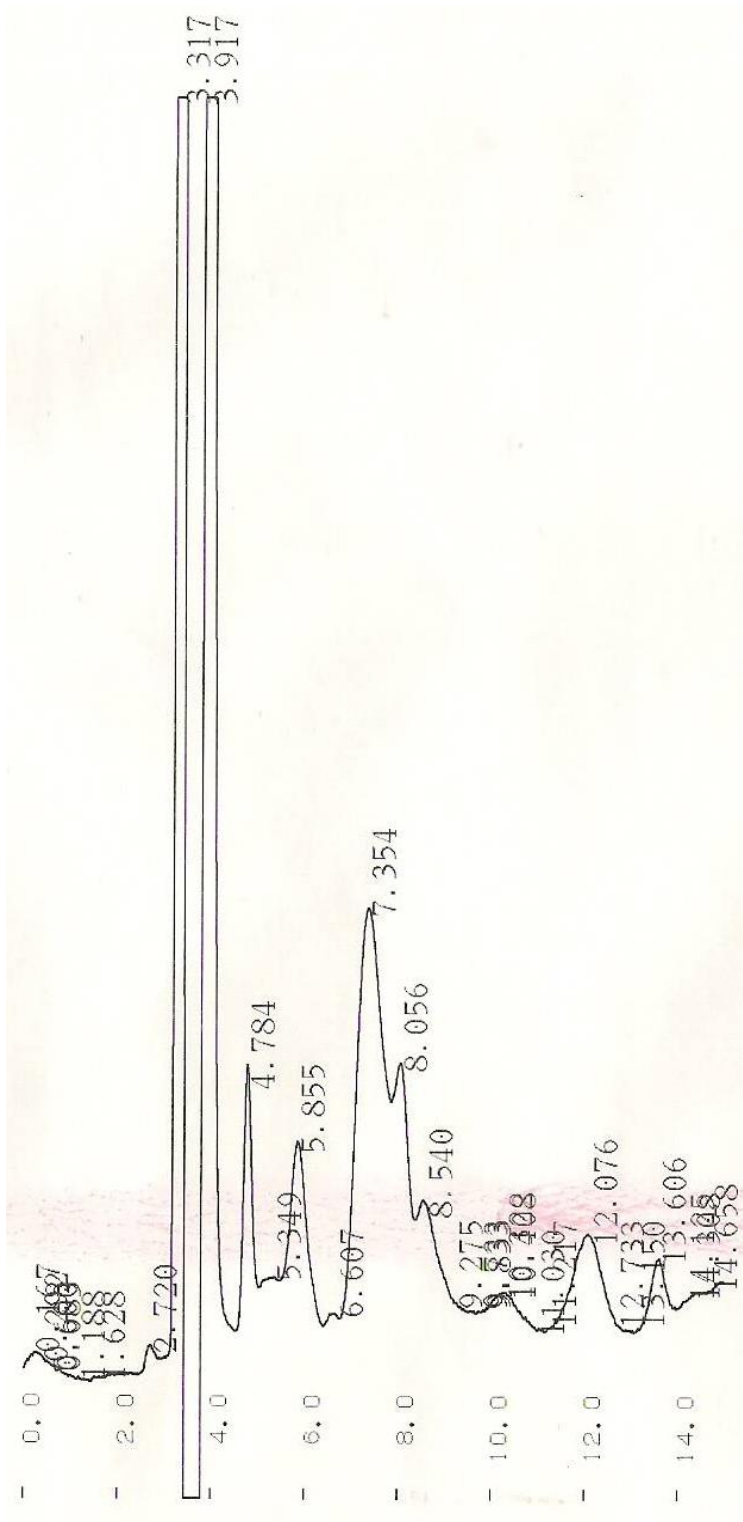
APPENDIX XVI

Chromatogram for a sample of kales from seed bed station 3.



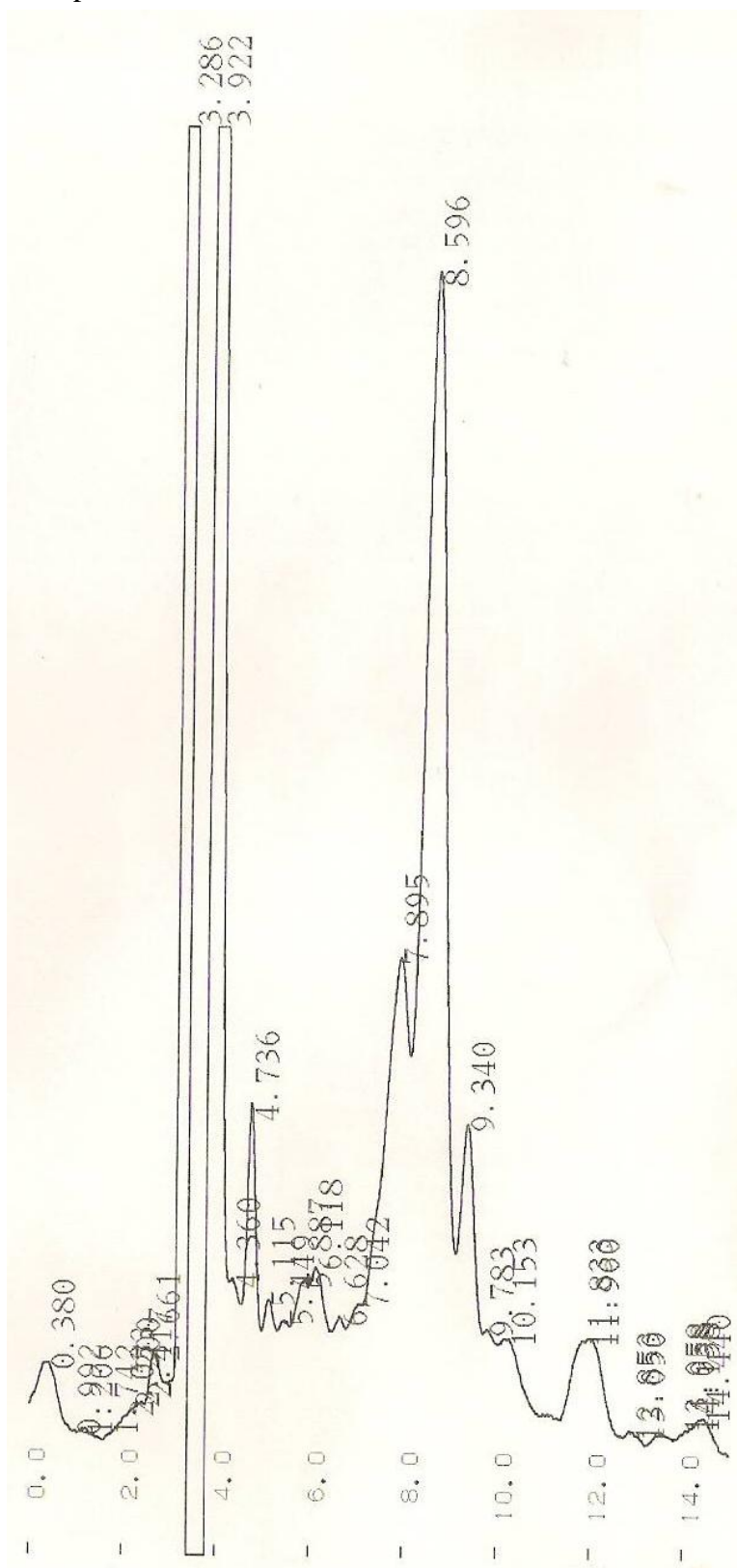
APPENDIX XVII

Chromatogram for a sample of spinach sprayed station 4.



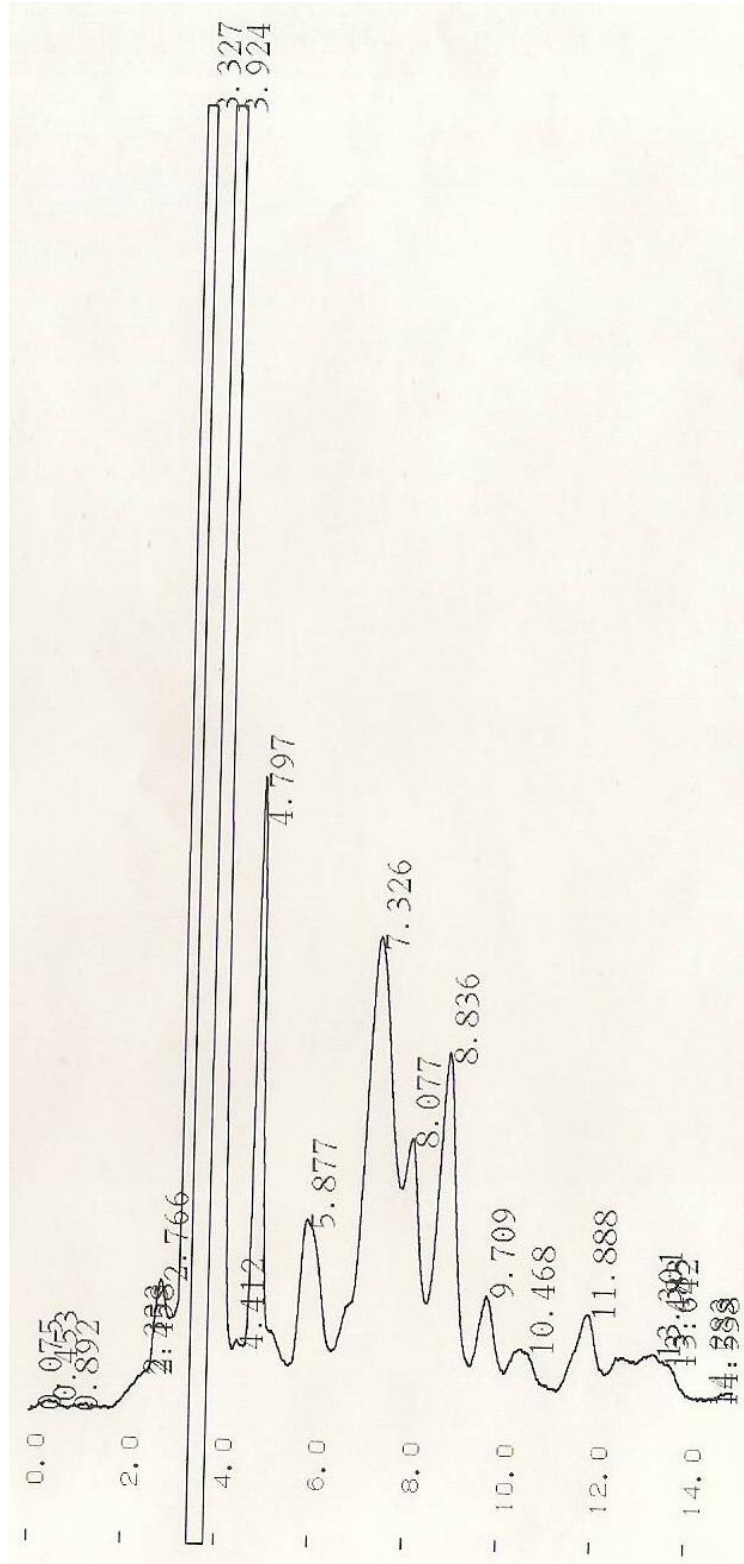
APPENDIX XVIII

Chromatogram for a sample of kales from isibania market.



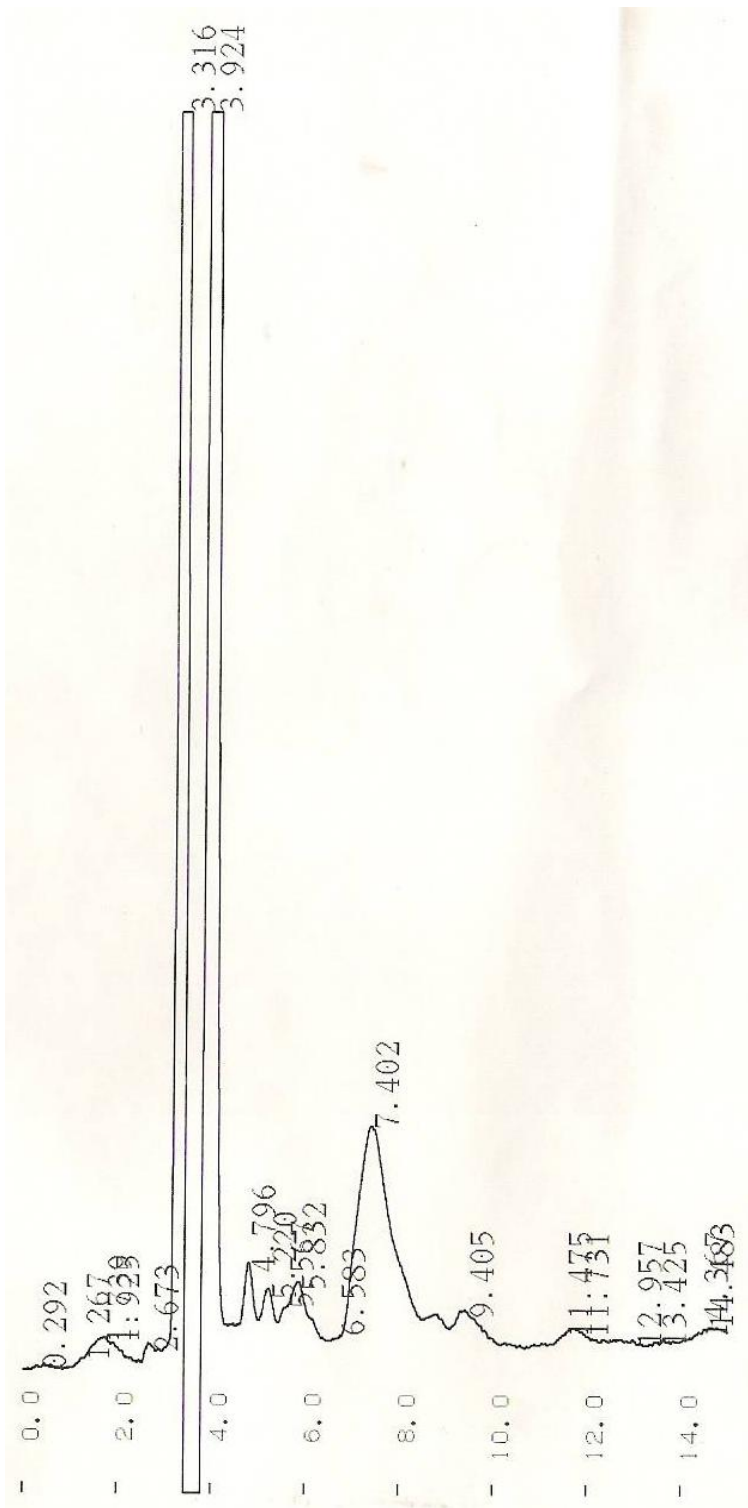
APPENDIX XIX

Chromatogram for a sample of kales sprayed station 3.



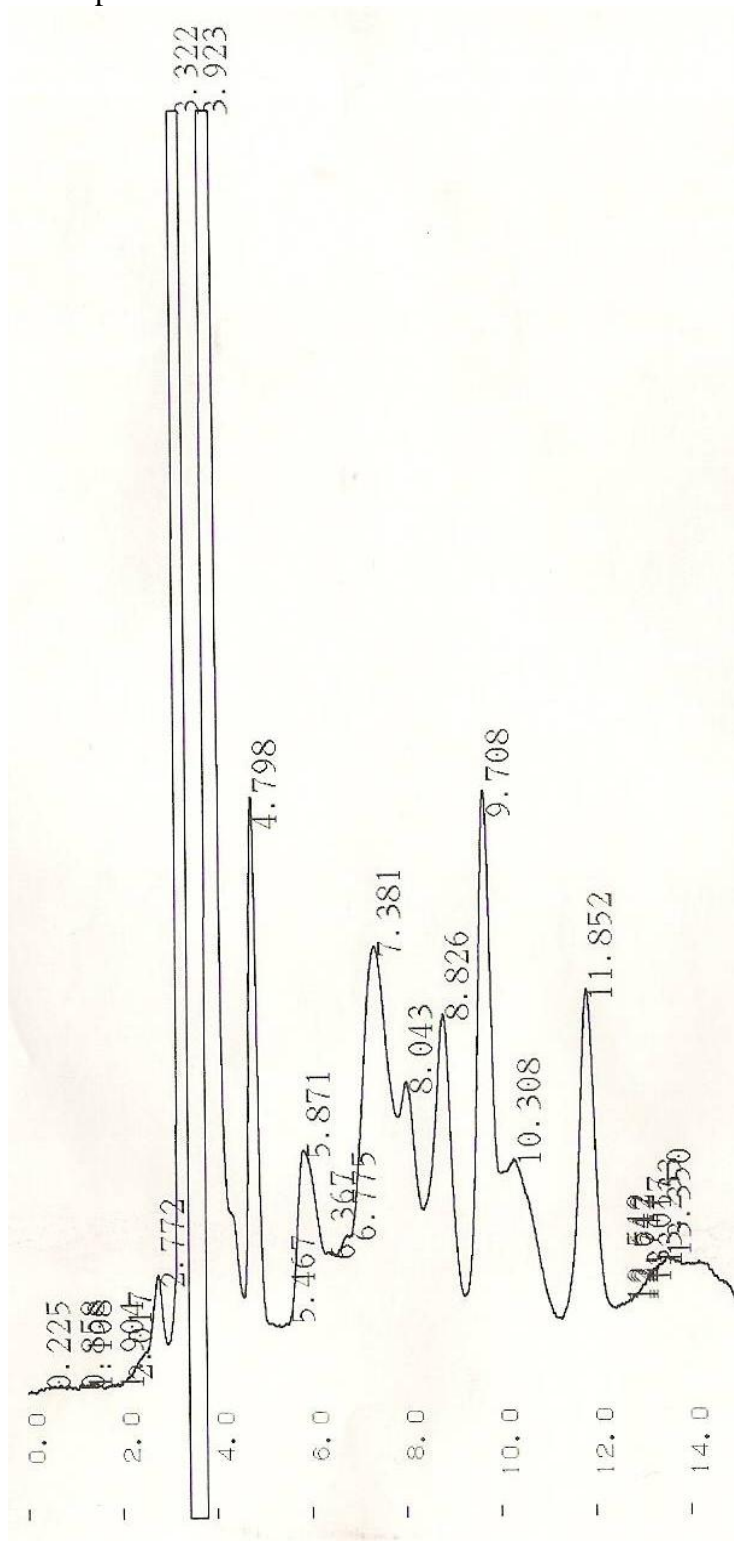
APPENDIX XX

Chromatogram for a sample of amaranthus from isibania market.



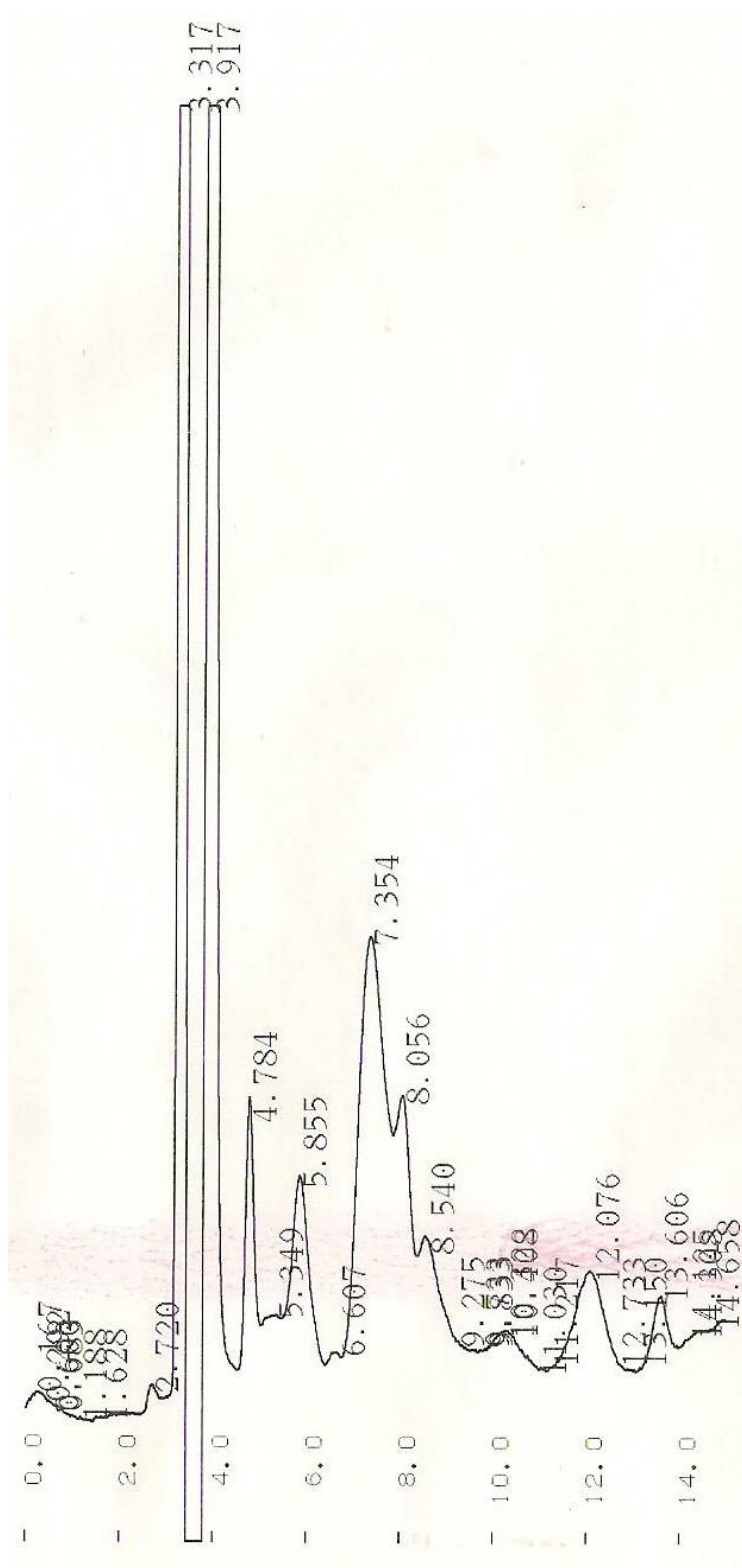
APPENDIX XXI

Chromatogram for a sample of kales from seed bed station 3.



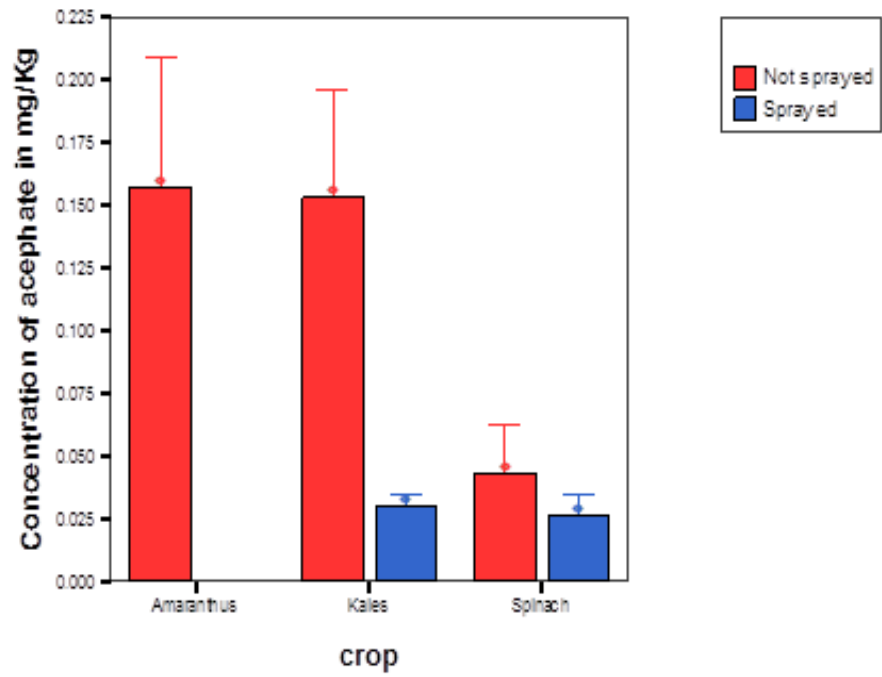
APPENDIX XXII

Chromatogram for a sample of spinach sprayed station 4.



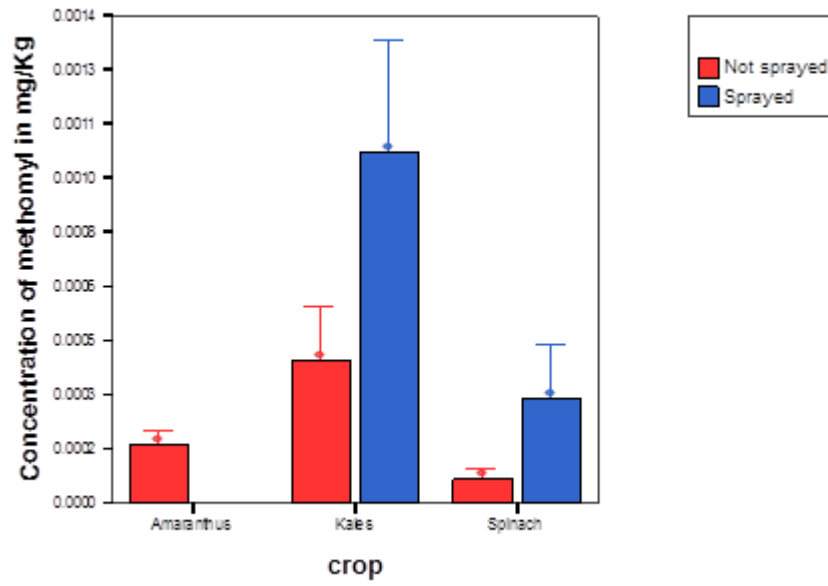
APPENDIX XXIII

Mean residue levels of acephate in the vegetables



APPENDIX XXIV

Mean residue levels for methomyl in the vegetables



APPENDIX XXV**RAW DATA FOR EXPERIMENTAL GARDEN**

DAY	CODE	CONC. OF ACEPHATE FOR SPRAYED	CONC. OF METHOMYL FOR SPRAYED	ACEPHATE CONC. (CONTROL)	METHOMYL CONC. (CONTROL)
2	A	0.0370	0.0037	0.0225	BDL
	B	0.0375	0.0035	0.0226	BDL
	C	0.0365	0.0038	0.0227	BDL
3	A	0.0318	0.0061	0.0201	BDL
	B	0.0320	0.0058	0.0200	BDL
	C	0.0316	0.0060	0.0203	BDL
4	A	0.0309	0.0055	0.0182	BDL
	B	0.0313	0.0054	0.0185	BDL
	C	0.0306	0.0056	0.0180	BDL
5	A	0.0306	0.0044	0.0162	BDL
	B	0.0309	0.0040	0.0161	BDL
	C	0.0303	0.0047	0.0163	BDL
6	A	0.0266	0.0033	0.0155	BDL
	B	0.0270	0.0035	0.0153	BDL
	C	0.0262	0.0031	0.0152	BDL
7	A	0.0259	0.0032	0.0148	BDL
	B	0.0255	0.0035	0.0149	BDL
	C	0.0263	0.0030	0.0147	BDL
8	A	0.0259	0.0031	0.0135	BDL
	B	0.0260	0.0030	0.0133	BDL
	C	0.0258	0.0032	0.0134	BDL

APPENDIX XXVI

RAW DATA FOR EXPERIMENTAL GARDEN

DAY	CODE	CONC. OF ACEPHATE FOR SPRAYED	CONC. OF METHOMYL FOR SPRAYED	ACEPHATE CONC. (CONTROL)	METHOMYL CONC. (CONTROL)
9	A	0.0252	0.0026	0.0128	BDL
	B	0.0250	0.0032	0.0125	BDL
	C	0.0254	0.0029	0.0127	BDL
10	A	0.0249	0.0024	0.0124	BDL
	B	0.0253	0.0029	0.0126	BDL
	C	0.0245	0.0026	0.0123	BDL
11	A	0.0228	0.0025	0.0119	BDL
	B	0.0226	0.0027	0.0118	BDL
	C	0.0230	0.0026	0.0115	BDL
12	A	0.0212	0.0028	0.0108	BDL
	B	0.0214	0.0022	0.0105	BDL
	C	0.0210	0.0025	0.0107	BDL
13	A	0.0207	0.0021	BDL	BDL
	B	0.0210	0.0027	BDL	BDL
	C	0.0204	0.0024	BDL	BDL
14	A	0.0180	0.0004	BDL	BDL
	B	0.0178	0.0005	BDL	BDL
	C	0.0182	0.0004	BDL	BDL
15	A	0.0115	0.0002	BDL	BDL
	B	0.0113	0.0004	BDL	BDL
	C	0.0117	0.0003	BDL	BDL

APPENDIX XXVII

RAW DATA FOR SAMPLES

code	crop			ace	meth	season
ST1	Amaranthus	Feb	Seedbed	0.19	0.0006	dry
ST1	Amaranthus	Feb	Seedbed	0.2	0.0006	dry
ST1	Amaranthus	Feb	Seedbed	0.2	0.0006	dry
ST2	Amaranthus	Feb	Market	0	0	dry
ST2	Amaranthus	Feb	Market	0	0	dry
ST2	Amaranthus	Feb	Market	0	0	dry
ST3	Amaranthus	Feb	Seedbed	0	0.0005	dry
ST3	Amaranthus	Feb	Seedbed	0	0.0006	dry
ST3	Amaranthus	Feb	Seedbed	0	0.0006	dry
ST4	Amaranthus	Feb	Seedbed	0.97	0.0002	dry
ST4	Amaranthus	Feb	Seedbed	1.1	0.0002	dry
ST4	Amaranthus	Feb	Seedbed	1.07	0.0002	dry
ST1	Amaranthus	Jan	Seedbed	0.19	0	dry
ST1	Amaranthus	Jan	Seedbed	0.2	0	dry
ST1	Amaranthus	Jan	Seedbed	0.2	0	dry
ST2	Amaranthus	Jan	Market	0.16	0	dry
ST2	Amaranthus	Jan	Market	0.17	0	dry
ST2	Amaranthus	Jan	Market	0.17	0	dry
ST5	Amaranthus	March	Market	0.02	0	Wet
ST5	Amaranthus	March	Market	0.02	0	Wet
ST5	Amaranthus	March	Market	0.02	0	Wet
ST1	Amaranthus	March	Seedbed	0.01	0	Wet
ST1	Amaranthus	March	Seedbed	0.01	0	Wet

APPENDIX XXVIII

RAW DATA FOR SAMPLES

ST1	Amaranthus	March	Seedbed	0.01	0	Wet
ST3	Amaranthus	March	Seedbed	0.02	0.0003	Wet
ST3	Amaranthus	March	Seedbed	0.03	0.0003	Wet
ST3	Amaranthus	March	Seedbed	0.02	0.0003	Wet
ST4	Amaranthus	March	Seedbed	0.03	0	Wet
ST4	Amaranthus	March	Seedbed	0.03	0	Wet
ST4	Amaranthus	March	Seedbed	0.03	0	Wet
ST5	Amaranthus	May	Market	0.08	0.0002	Wet
ST5	Amaranthus	May	Market	0.08	0.0002	Wet
ST5	Amaranthus	May	Market	0.08	0.0002	Wet
ST2	Amaranthus	May	Market	0.02	0	Wet
ST2	Amaranthus	May	Market	0.02	0	Wet
ST2	Amaranthus	May	Market	0.02	0	Wet
ST3	Amaranthus	May	Seedbed	0.04	0.0002	Wet
ST3	Amaranthus	May	Seedbed	0.03	0.0002	Wet
ST3	Amaranthus	May	Seedbed	0.04	0.0002	Wet
ST1	Kales	Feb	Seedbed	0.03	0	dry
ST1	Kales	Feb	Seedbed	0.03	0	dry
ST1	Kales	Feb	Seedbed	0.03	0	dry
ST3	Kales	Feb	Seedbed	0.01	0.0004	dry
ST3	Kales	Feb	Seedbed	0.02	0.0004	dry
ST3	Kales	Feb	Seedbed	0.02	0.0004	dry
ST4	Kales	Feb	Seedbed	0	0	dry

APPENDIX XXIX

RAW DATA FOR SAMPLES

ST4	Kales	Feb	Seedbed	0	0	dry
ST4	Kales	Feb	Seedbed	0	0	dry
ST2	Kales	Feb	Market	0	0.0045	dry
ST2	Kales	Feb	Market	0	0.0045	dry
ST2	Kales	Feb	Market	0	0.0046	dry
ST5	Kales	Jan	Market	0.01	0	dry
ST5	Kales	Jan	Market	0.01	0	dry
ST5	Kales	Jan	Market	0.01	0	dry
ST1	Kales	Jan	Seedbed	0.4	0.001	dry
ST1	Kales	Jan	Seedbed	0.39	0.0011	dry
ST1	Kales	Jan	Seedbed	0.43	0.0011	dry
ST2	Kales	Jan	Market	0.76	0	dry
ST2	Kales	Jan	Market	0.82	0	dry
ST2	Kales	Jan	Market	0.86	0	dry
ST4	Kales	Jan	Seedbed	0.47	0	dry
ST4	Kales	Jan	Seedbed	0.5	0	dry
ST4	Kales	Jan	Seedbed	0.5	0	dry
ST4	Kales	Jan	Sprayed	0.07	0.0042	dry
ST4	Kales	Jan	Sprayed	0.06	0.0046	dry
ST4	Kales	Jan	Sprayed	0.06	0.0052	dry
ST5	Kales	March	Market	0.02	0	Wet
ST5	Kales	March	Market	0.03	0	Wet
ST5	Kales	March	Market	0.03	0	Wet

APPENDIX XXX

RAW DATA FOR SAMPLES

ST1	Kales	March	Seedbed	0.03	0.0002	Wet
ST1	Kales	March	Seedbed	0.03	0.0002	Wet
ST1	Kales	March	Seedbed	0.03	0.0002	Wet
ST2	Kales	March	Market	0.02	0.0032	Wet
ST2	Kales	March	Market	0.02	0.0033	Wet
ST2	Kales	March	Market	0.02	0.0036	Wet
ST3	Kales	March	Seedbed	0.01	0	Wet
ST3	Kales	March	Seedbed	0.01	0	Wet
ST3	Kales	March	Seedbed	0.01	0	Wet
ST4	Kales	March	Seedbed	0	0	Wet
ST4	Kales	March	Seedbed	0	0	Wet
ST4	Kales	March	Seedbed	0	0	Wet
ST1	Kales	March	Sprayed	0.03	0.0004	Wet
ST1	Kales	March	Sprayed	0.03	0.0004	Wet
ST1	Kales	March	Sprayed	0.04	0.0004	Wet
ST2	Kales	March	Market	0.02	0	Wet
ST2	Kales	March	Market	0.02	0	Wet
ST2	Kales	March	Market	0.02	0	Wet
ST3	Kales	March	Sprayed	0.03	0.0002	Wet
ST3	Kales	March	Sprayed	0.03	0.0002	Wet
ST3	Kales	March	Sprayed	0.03	0.0002	Wet
ST4	Kales	March	Sprayed	0	0	Wet
ST4	Kales	March	Sprayed	0	0	Wet

APPENDIX XXXI

RAW DATA FOR SAMPLES

ST4	Kales	March	Sprayed	0	0	Wet
ST5	Kales	May	Market	0.04	0	Wet
ST5	Kales	May	Market	0.04	0	Wet
ST5	Kales	May	Market	0.04	0	Wet
ST2	Kales	May	Market	0.01	0	Wet
ST2	Kales	May	Market	0.01	0	Wet
ST2	Kales	May	Market	0.01	0	Wet
ST3	Kales	May	Seedbed	0.01	0	Wet
ST3	Kales	May	Seedbed	0.01	0	Wet
ST3	Kales	May	Seedbed	0.01	0	Wet
ST1	Kales	May	Sprayed	0.03	0	Wet
ST1	Kales	May	Sprayed	0.03	0	Wet
ST1	Kales	May	Sprayed	0.03	0	Wet
ST2	Kales	May	Market	0.05	0.0001	Wet
ST2	Kales	May	Market	0.04	0.0001	Wet
ST2	Kales	May	Market	0.04	0.0001	Wet
ST3	Kales	May	Sprayed	0	0.0004	Wet
ST3	Kales	May	Sprayed	0	0.0004	Wet
ST3	Kales	May	Sprayed	0	0.0004	Wet
ST4	Kales	May	Sprayed	0.07	0	Wet
ST4	Kales	May	Sprayed	0.08	0	Wet
ST4	Kales	May	Sprayed	0.08	0	Wet
ST1	Spinach	Feb	Seedbed	0	0	dry

APPENDIX XXXII

RAW DATA FOR SAMPLES

ST1	Spinach	Feb	Seedbed	0	0	dry
ST1	Spinach	Feb	Seedbed	0	0	dry
ST4	Spinach	Feb	Seedbed	0	0.0004	dry
ST4	Spinach	Feb	Seedbed	0	0.0004	dry
ST4	Spinach	Feb	Seedbed	0	0.0004	dry
ST1	Spinach	Jan	Seedbed	0.26	0	dry
ST1	Spinach	Jan	Seedbed	0.26	0	dry
ST1	Spinach	Jan	Seedbed	0.26	0	dry
ST5	Spinach	March	Market	0	0.0001	Wet
ST5	Spinach	March	Market	0	0.0001	Wet
ST5	Spinach	March	Market	0	0.0001	Wet
ST1	Spinach	March	Seedbed	0	0.0001	Wet
ST1	Spinach	March	Seedbed	0	0.0001	Wet
ST1	Spinach	March	Seedbed	0	0.0001	Wet
ST3	Spinach	March	Seedbed	0	0	Wet
ST3	Spinach	March	Seedbed	0	0	Wet
ST3	Spinach	March	Seedbed	0.01	0	Wet
ST4	Spinach	March	Seedbed	0.03	0	Wet
ST4	Spinach	March	Seedbed	0.03	0	Wet
ST4	Spinach	March	Seedbed	0.03	0	Wet
ST3	Spinach	March	Sprayed	0.01	0	Wet
ST3	Spinach	March	Sprayed	0.01	0	Wet
ST3	Spinach	March	Sprayed	0.01	0	Wet

APPENDIX XXXIII

RAW DATA FOR SAMPLES

ST4	Spinach	March	Sprayed	0.01	0.0009	Wet
ST4	Spinach	March	Sprayed	0.01	0.0009	Wet
ST4	Spinach	March	Sprayed	0.01	0.0009	Wet
ST3	Spinach	May	Seedbed	0.01	0	Wet
ST3	Spinach	May	Seedbed	0.01	0	Wet
ST3	Spinach	May	Seedbed	0.01	0	Wet
ST3	Spinach	May	Sprayed	0.06	0	Wet
ST3	Spinach	May	Sprayed	0.06	0	Wet
ST3	Spinach	May	Sprayed	0.06	0	Wet