

**METHOD OPTIMIZATION AND DETERMINATION OF SELECTED
ORGANOPHOSPHATE AND CARBAMATE PESTICIDE RESIDUES IN THE RICE
(*Oryza Sativa*) GROWING REGION OF MWEA, KIRINYAGA COUNTY, KENYA**

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**A Thesis Submitted in partial Fulfilment of the Requirements for award Degree of Master
of Science in Chemistry of Jomo Kenyatta University of Agriculture and Technology**

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DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree award.

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DEDICATION

To my family, who offered me unconditional love and support throughout the study.

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LIST OF ABBREVIATIONS AND ACRONYMS

3-HC	3-Hydroxycarbofuran
CB	Carbofuran
EPA	Environmental Protection Agency
EU	European Union
GC-MS	Gas Chromatography - Mass Spectrometry
GDP	Gross Domestic Product
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LPME	Liquid Phase Microextraction
NEMA	National Environmental Management Authority
OP	Organophosphate
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
WHO	World Health Organization

ABSTRACT

Carbamate and organophosphate pesticides have been widely used in rice production in the Mwea region, Kirinyaga County, Kenya for the control and elimination of pests. This region is found in central Kenya and is the largest rice irrigation scheme in Kenya accounting for over 70% of the rice produced in Kenya. Concern over the presence of pesticide residues within the farmlands have arisen. Exposure to pesticide residues is known to cause myriad of health effects to both human and animal life such as endocrine disruption or even death. The main objective of the study was to investigate for the residues of the highly toxic pesticide carbofuran, its major metabolite 3-hydroxycarbofuran and two organophosphates- diazinon and fenitrothion within the agricultural region of Mwea, Kenya. Soil, water from within the rice paddies and from two major channels supplying the paddies with water as well as rice plant samples were collected for analysis during the month of June, 2013 (n=34 in total). Extraction of pesticides from water was carried out using the solid phase extraction method. Soil and rice samples were air-dried thereafter pesticides extracted using organic solvents employing the Soxhlet method of extraction. The extracts were analysed using gas chromatograph –mass spectrometer. Prior to sample analysis, method validation and optimization studies were carried out. A test on the efficiency of two columns, a fast GC column- DB-XIb and a conventional column–Rtx-5MS resulted in the fast GC column giving better resolution and lower limit of detections (LOD) values. Carbofuran and its metabolites were below the detection limit ($0.38\mu\text{g/L}$) in all of the samples from the agricultural farmlands study site. However, the two organophosphate pesticides under study were detected in water sampled from the irrigation paddies and in soil samples. Fenitrothion was detected in soil from two sections of the study site (Karaba A and B) at levels of 0.06 ± 0.012 and 0.11 ± 0.054 mg/kg respectively. Diazinon was detected in both a single soil sample and single paddy water sample (from Wamumu A) at 0.24 ± 0.033 mg/kg and 0.19 ± 0.065 mg/L respectively. All the pesticide levels detected were above maximum acceptable total and individual levels of contamination, as set by the European Union (0.5 and 0.1 $\mu\text{g/L}$, respectively). The soil pH varied from 5.29-5.63 and was considered to be strongly acidic while water pH varied from 6.84-5.46. From the study, a simple and clear method for the analysis of carbamate and organophosphate pesticides was developed. pH was observed to be a vital

physico-chemical parameter in determining the availability of carbamate pesticides in the environment and that carbamate pesticides residues tend to more available in acidic environment.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 BACKGROUND

There is intensive use of pesticides by farmers to increase crop production in Kenya. Mwea region in Kirinyaga County is an agricultural region where heavy rice production is carried out. In order to meet the demand for quantity and quality in agricultural products farmers use different types of pesticides to control and eliminate parasites, insects and fungal diseases. This, in turn, has resulted in entry of the chemical contaminants into the furrows, channels, canals and streams within the rice farms leading to pollution of the rivers and crops by pesticides (Nhapi *et al.*, 2011). Rice is the only major crop that can be grown in the standing water in vast areas of flat, low-lying tropical soils and is uniquely adapted for growth in submerged conditions. Rice is grown in the tropical and subtropical regions of most continents. It is cultivated under widely differing conditions because of the great cultivar diversity (NRDS, 2009). Rice cultivation was introduced in Kenya, from Asia, in 1907. In Kenya, it is currently the third most important cereal crop after maize and wheat. About 80% of the rice grown in Kenya is from Government-established irrigation schemes, while the remaining 20% is produced under (unassisted) rain-fed conditions (NRDS, 2009). The national rice consumption is estimated at 300,000 metric while local production is estimated at between 33,000 and 50,000 metric tons. The rice is mainly produced by small-scale farmers. Organophosphates and carbamate pesticide are used in the area for pesticide control. Pesticides can remain as residues in soil, water or on surfaces from where human beings are constantly exposed (Frederick, 2005). Depending on the type of pesticide applied, the vapours and residues may also drift or volatilize off the treatment site thereby contaminating air, soil and vegetation and increasing the potential for human exposure. The main routes of pesticide exposure are ingestion, inhalation and dermal where both acute and chronic poisoning with pesticides has been reported. However exposure of human to pesticides is mainly from residues in food, where the level of exposure depends on both the quantity of food consumed and the level of the residues (Andrew, 2002). As a result of extensive use of chemicals, there was need to determine the levels of these pesticides in Mwea, Kirinyaga County.

1.2 Classes of pesticides

The U.S. Environmental Protection Agency (US EPA, 2002) defines pesticides generally as any substance intended for preventing, destroying, repelling, or mitigating any pest while Kamrin (1997) defined it as any compound developed and used to stop the growth or end the life of a wide range of living organisms that man considers as pest. Jørgen (2004) defines pesticide residues as substances in food, forage or the environment that are present because of use of pesticides. The residues may include derivatives such as degradation products metabolites, reaction products metabolites and impurities that may have toxicological significance. Major classes of pesticides include organochlorines, organophosphates, organochlorines and pyrethroids

1.2.1 Organophosphate pesticides

This is a phosphorous-rich organic compound that contains a halide compound. These pesticides affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter (Jamal *et al.*, 2002). Most organophosphates (OP's) used in rice production are insecticides and are active against a broad spectrum of insects (Chowudhry *et al.*, 2012). Their effects on insects are similar to their effects on humans. Organophosphates are classified by the US-EPA as highly or moderately toxic. OP's of primary concern include: azinphos-methyl, chlorpyrifos, diazinon, dichlorvos, dimethoate, fenitrothion, ethephon, malathion, methamidophos, naled, and oxydemeton-methy (PAN-UK, 2010).

1.2.1.1 Diazinon

Diazinon is an organophosphate insecticide used in agriculture to control insects on fruit, vegetable, nut and field crops. It is also used to control agricultural soil-dwelling insects, and is applied as a sheep dip to control ectoparasites such as sheep scab and blow fly strike (Tomlin, 1997). Diazinon products are formulated as dusts, granules, liquids, concentrates, microencapsulations, wettable powders, seed dressings and impregnated materials. Diazinon used mostly by Mwea farmers is purchased under trade name of 'Neocidal'. It has water of solubility of 0.04 g/L at 20 °C and a molecular weight 304 g/mol. It is listed by the US EPA as

moderately toxic to human however; it is known to be highly toxic to bees and other beneficial insects in case of acute to exposure (US EPA, 2006). Figure 1.1 shows the molecular structure of diazinon pesticide.

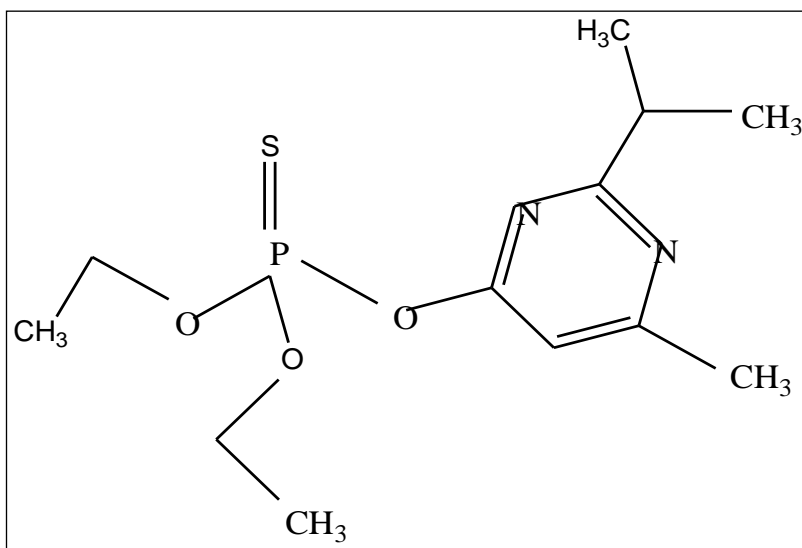


Figure 1.1: Molecular structure of diazinon

1.2.1.2 Fenitrothion

Fenitrothion (C₉H₁₂NO₅PS) is a contact organophosphate insecticide and selective acaricide of low ovicidal properties - a highly odorous broad spectrum insecticide and is ideal for use as a repellent spray. It is effective against a wide range of pests such as coffee leaf miners, locusts and rice stem borers. Fenitrothion is non-systemic, and non-persistent (Kidd and James, 1994). In rice, fenitrothion is an effective product for the control of the damaging rice bug especially during the maturing stage. It is identified locally under trade name of 'sumithion super'. It is of moderate toxicity to fish and birds, has a molecular weight of 277.25 g/mol and water solubility of 30 mg/l at 20⁰C (EXTOXNET, 1995).Figure 1.2 shows the molecular structure of fenitrothion pesticide

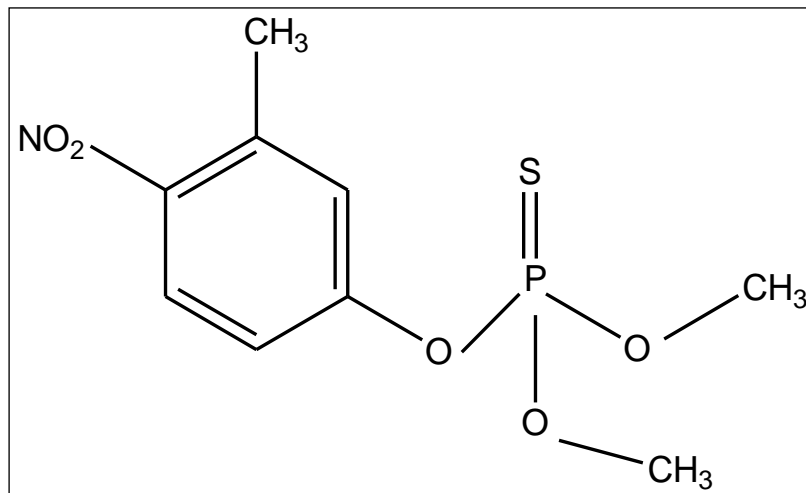


Figure 1.2: Molecular structure of fenitrothion

1.2.2 Carbamate pesticides

Carbamate pesticides are derived from carbamic acid and kill insects in homes, gardens and agriculture. Like the organophosphates, their mode of action is inhibition of cholinesterase enzymes, affecting nerve impulse transmission (Zapletal, 2001). Most of the carbamates are extremely toxic to bees, and precautions must be taken to avoid exposure to foraging bees or parasitic wasps (Ameno *et al.*, 2001). Some of the carbamates are translocated within plants, making them an effective systemic treatment (Crocker, 2005). The carbamate's principal route of entry is either by inhalation or ingestion or secondarily by the dermal route. Dermal exposure tends to be the less toxic route than inhalation or ingestion (Pesticide Toxicity Profile, 2013)

1.2.2.1 Carbofuran

Carbofuran (C₁₂H₁₅NO₃) is a broad-spectrum systemic insecticide, nematicide, and acaricide (Richards, 2011). Carbofuran is highly mobile in soils and appears in waters because of its high solubility. It has a high potential for underground water contamination of aquifers via leaching from treated fields, and also from surface waters which enter as a result of runoff from treated fields (Lalah & Wandiga, 1996). It has a molar mass of 221.3 g/mol and water solubility of 700 mg/l. The main metabolites of carbofuran which have been found in various matrices are 3-hydroxycarbofuran and 3-ketocarbofuran. Carbofuran is classified by WHO as extremely

hazardous to humans (WHO 1A). Figure 1.3 and 1.4 shows the molecular structure of carbofuran and 3-hydroxycarbofuran compounds.

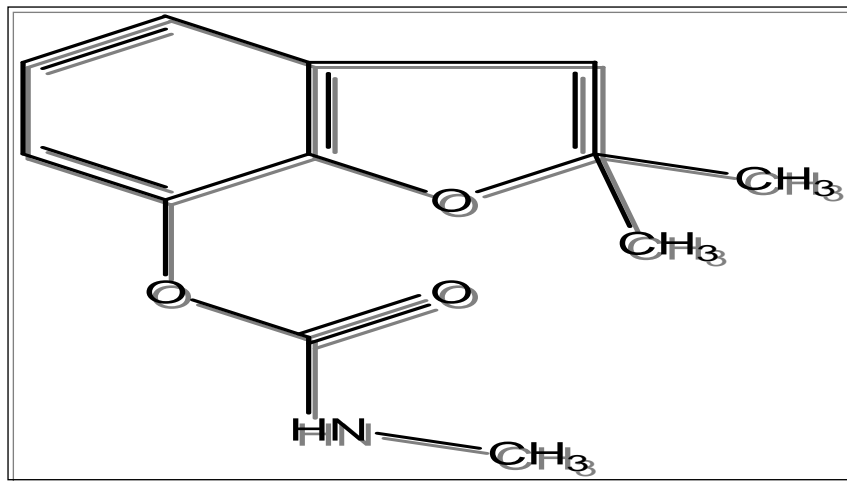


Figure 1.3: Molecular structure of carbofuran

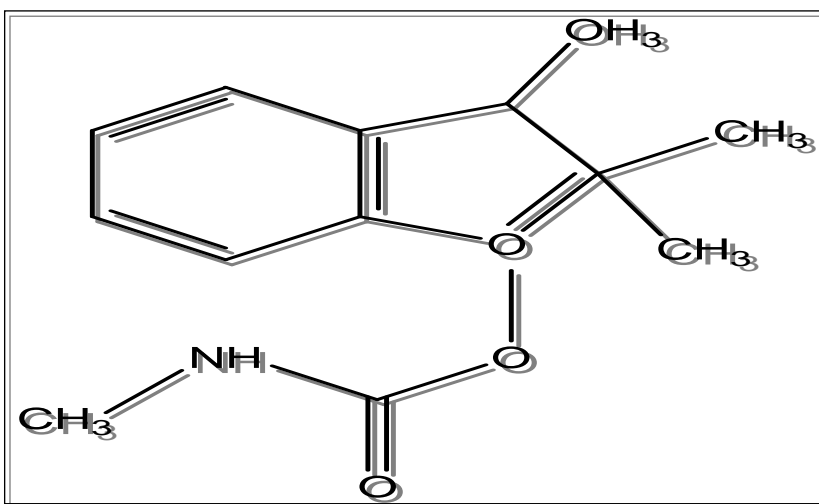


Figure 1.4: Molecular structure of 3-hydroxycarbofuran

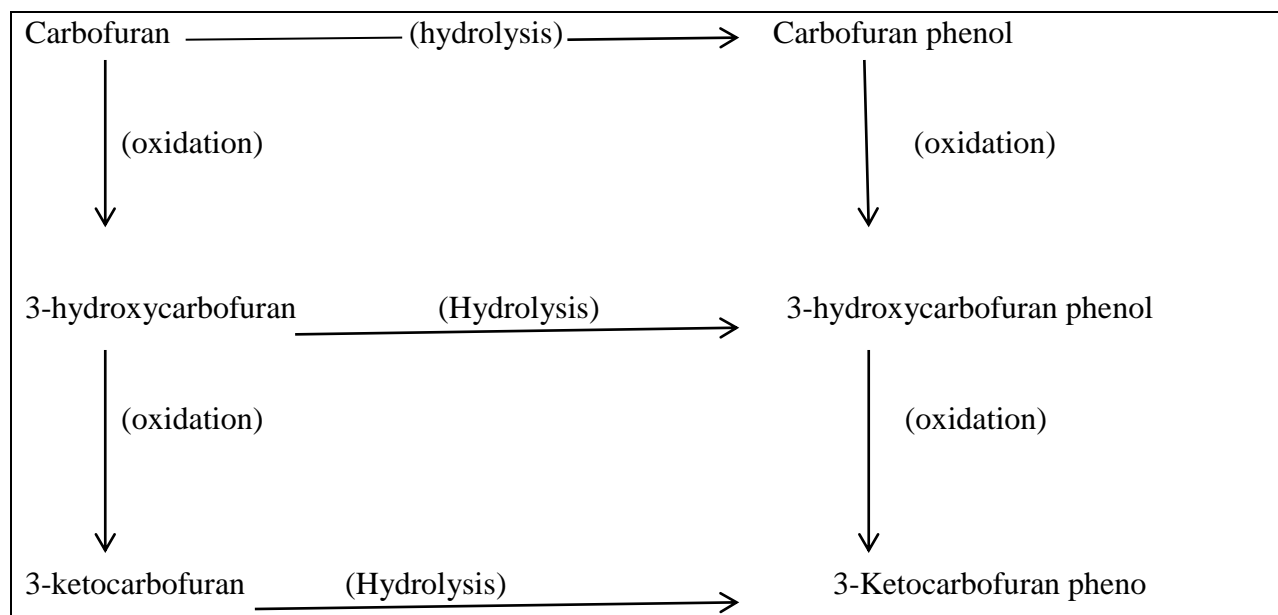


Figure 1.5: Degradation pathways of carbofuran to metabolites by oxidation and hydrolysis

1.2.3 Organochlorines, pyrethroids

Organochlorine compounds are synthetic organic insecticides that contain carbon, hydrogen, chlorine and sometimes oxygen (Afful *et al.*, 2010). Contamination of the environment by

organochlorine pesticides is found in many places due to the fact that these compounds are and food web long after being applied. Pyrethroids are organic synthetic insecticides that are widely used for the protection of crops and food storage against insects and acarids (Parilla *et al.*, 2005)

1.2.4 Pesticide use in rice growing

Agriculture accounts for about 24% of Kenya's GDP with an estimated 75% of the population depending on the sector either directly or indirectly. As an agricultural economy, Kenya's demand for pesticides is relatively high. The import demand is further fuelled by regional consumption in land locked countries like Uganda, Rwanda and Burundi. Kenya imports approximately 7,000 metric tons of pesticides worth billions of Kenya shillings (US\$ 50 million) (Nyakundi *et al.*, 2012). These pesticides are an assortment of insecticides, fungicides, herbicides fumigants, rodenticides, growth regulators, defoliators, proteins, surfactants and wetting agents. Of the total pesticide imports, insecticides account for about 40% in terms of volume (2,900 metric tons) and 50% of the total cost of pesticide imports (Nyakundi *et al.*, 2012).

The insecticides used in rice production belong to the extremely hazardous category I and 11 chemicals making individuals exposed to them very susceptible to pesticide-related illnesses. The concern about pesticide use in rice is majorly about the overly intensive use. Unsafe application techniques and other unsafe pesticide use practices that harm rice workers also come in too (Pingali *et al.*, 1991).

Kenya-based research has revealed environmental contamination by pesticides and the potential for adverse environmental effects from agricultural uses. For example, pesticide runoff from neighbouring horticultural farms into the Lake Naivasha ecosystem has been documented (Kaoga *et al.*, 2013). Otieno (2010) and co-workers were able to detect carbofuran and 3-hydroxycarbofuran, one of its primary metabolites, in farmlands at concentration ranging from 0.010–1.009 mg/kg in dry surface soil and 0.005–0.495 mg/L in water samples from two rivers flowing through the farms in Isisolo where Furadan (the trade name of a carbofuran-based product) had been used extensively. In lower Nyando-Nyanza Kenya, within Lake Victoria basin, a rice growing region too, diazinon and dimethoate residues of concentration range 0.56

$\mu\text{g}/\text{kg}$ to $1.08 \mu\text{g}/\text{kg}$ have reportedly been detected in soil (Musa *et al.*, 2011). Odino and Ogada (2008) in 2009 conducted a field study in Bunyala rice -Western Kenya irrigation scheme and determined that carbofuran pesticide was deliberately being used to kill wild birds for human consumption. Excessive use of pesticides in Kenyan horticultural industry has also been reported by Okado (2001) and Jaffee (2003) suggesting that many farmers used pesticides indiscriminately, in some cases, applying pesticides meant for other crops (such as coffee) on fresh vegetables.

In South-Tanzania, Ngowi *et al.*, (2007) found out that the small-scale farmers grow crops which include include tomatoes, cabbages, rice and maize using many types of pesticides to control pests and diseases that attack these crops. The types of pesticides used by the farmers in the study areas were insecticides (59%), fungicides (29%) and herbicides (10%) with the remaining 2% being rodenticides. About a third of the farmers applied pesticides in mixtures. In all cases there were no specific instructions either from the labels or extension workers regarding these tank mixtures. This is a trend observed in many African countries in crop farming. Sixty eight percent of farmers reported having felt sick after routine application of pesticides.

Further away, in Vietnam over 95 per cent of rice farmers applied at least one type of pesticide during the growing season and the mean number of sprays in Vietnam is seven (PANUPS, 1995). Over 90% of pesticides sprayed were insecticides, approximately half being organophosphates, including methyl parathion, diazinon, monocrotophos, fenitrothion and methamidophos. Of the pesticides used, nearly 20 percent were classified by the World Health Organization as extremely hazardous (PANUPS, 1995).

Table 1.1 shows a number of pesticides that are used in rice production. All these pesticides have been registered with Pest Control Products Board (PCPB) with carbofuran being one of the most highly restricted pesticides listed in the table in Kenya (PCPB, 2015 and EURL, 2011)

Table 1.1: Pesticides authorized for use in rice growing by some EU states and Indian government

Insecticide		Herbicides		Fungicides	
Insecticides	WHO classification	Herbicides	WHO classification	Fungicide	WHO classification
Alpha-cypermethrine	Harzadous	Azimsulfuron	Harzadous	Propiconazole	Harzadous
Pirimiphos-methyl	Harzadous	Oxadiazon	Harzadous		
Fenitrothion	Harzadous	Bentazone	Harzadous		
Carbofuran	Extremely harzadous	Glyphosate	Harzadous		
Chlorpyriphos	Harzadous	Benthiocarb			
Methyl Bromide	Extremely harzadous				
Diazinon	Harzadous				

1.2.5 Sources of pesticides in the environment

Miller (2004) documented that over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target organisms, air, water and bottom sediments. Studies have shown unsafe storage, handling, application and disposal of pesticides increases the risk of incidental exposure and contamination of water canals and ducts (Maumbe & Swinton, 2003). Furthermore, toxic chemicals used in rice paddies are often not confined to them. During heavy rains, rice fields often overflow paddy boundaries contaminating surrounding soil and water (Lalah & Wandiga, 1996). According to Mukherjee *et al.*, (1999) the principal sources of pesticide residues in crops, food, animals, soil, water and almost all food commodities are:

- i. Carry-over from insecticide application to soil or to growing crops.
- ii. Leaching of pesticides (herbicides) or insecticides into ground water.
- iii. Drift of the pesticides from adjacent field.
- iv. Translocation of soil applied pesticide into growing crops.

- v. Disposal of pesticides in streams, rivers and lakes.
- vi. Effluents of pesticide industry in rivers and streams, and into soil which may be translocated in crops.

1.2.6 Toxicological effects of pesticides on human

In spite of stringent regulations by international and national regulatory agencies, reports of pesticide residues in human food, both imported and home-produced, are numerous (Waugh & Padovan, 2004). Effects of exposure to pesticides can range from mild skin irritation to birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, and even coma or death (Lorenz, 2009). Over the years many human illnesses and deaths have occurred as a result of exposure to pesticides. Some of these are suicides, but most involve some form of accidental exposure to pesticides, particularly among farmers and spray operators in developing countries (Steve, 2009).

1.2.3 Environmental exposure of pesticides

1.2.3.1 Effect of pesticides on terrestrial environment

Pesticides can remain as residues in soil, water or on surfaces from where human beings are constantly exposed. Depending on the type of pesticide applied, the vapours and residues may also drift or volatilize off the treatment site thereby contaminating air, soil and vegetation and increasing the potential for human exposure (Frederick & Fischer, 2005). Ware and Whitcare, (2000) found out that pesticides have considerable adverse environmental effects, which may be extremely diverse: sometimes simple but often extremely lethal and complex. Some pesticides are highly specific and others broad spectrum; both types can affect terrestrial wildlife, soil, water systems, and humans.

Pesticides have had some of their most striking effects on birds, particularly those in the higher trophic levels of food chains, such as bald eagles, hawks, and owls (Steve, 2009). These birds are often rare, endangered, and susceptible to pesticide residues such as those occurring from the bioconcentration of organochlorine insecticides through terrestrial food chains (Eldridge, 2008).

Pesticides may kill grain- and plant-feeding birds, and the elimination of many rare species of ducks and geese has been reported. Populations of insect-eating birds such as partridges, grouse, and pheasants have decreased due to the loss of their insect food in agricultural fields through the use of insecticides (Kamrin, 1997).

1.2.3.2 Effects on the aquatic environment

Many of the organisms that provide food for fish are extremely susceptible to pesticides, so the indirect effects of pesticides on the fish food supply may have an even greater effect on fish populations. Some pesticides, such as pyrethroid insecticides, are extremely toxic to most aquatic organisms (Waugh & Padovan, 2004). It is evident that pesticides cause major losses in global fish production (Steve, 2009).

1.3 Alkaline hydrolysis

The pH of aquatic systems is an important indicator of the water quality and extent of pollution in the area. Most pesticide formulations such as dry flowables, emulsifiable concentrates, and wettable powders are designed to be diluted with water as the carrier. Certain pesticides undergo alkaline hydrolysis at pH greater than 7. The severity and the rate in which it occurs is dependent on the pesticide, the alkalinity of the water, the length of time the pesticide is in contact with the water, and the water temperature. Insecticides, particularly organophosphates and carbamates, are more susceptible to alkaline hydrolysis than other pesticides. In comparison, sulfonyleurea herbicides are more susceptible to acid hydrolysis at pH less than 6.0 (Dara and Juong, 2013).

1.4 Analytical Techniques

1.4.1 Sample extraction and enrichment techniques

Pesticide residues appear in environmental samples at trace levels, which are well beyond the detection capability of most available analytical techniques (Jones, 2005). Some of the techniques commonly used for extraction/preconcentration of pesticide residues from aqueous

matrices include liquid–liquid (LLE), solid phase (SPE) extraction, liquid phase micro extraction (LPME) and solid phase micro extraction (SPME).

1.4.1.1 Solid-phase Extraction

Solid-phase extraction is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties (Supelco, 1998). SPE has gradually replaced classical liquid–liquid extraction and become the most common sample-preparation technique in environmental areas. Generally, SPE consists of four steps: column preparation (prewash), sample loading (retention or sorption), column postwash, and sample desorption (elution or desorption). The prewash step is used to condition the stationary phase if necessary, and the optional column postwash is used to remove undesirable contaminants (Orawee & Tinnakorn, 2007). Usually, the compounds of interest are retained on the sorbent while interferences are washed away. Analytes are recovered via an elution solvent. In the study C18 SPE cartridges and florisil-magnesium silicate were employed. C18 is capable of retaining analytes over a wide range of polarity while florisil was used for sample cleanup. Florisil is often used for cleanup of soil extracts for pesticide analysis. Florisil, a form of magnesium silicate, is a normal-phase sorbent and is used to remove polar interferences from the extract prior to GC or GC-MS analysis. This sample clean up precaution reduce high background levels that result in difficult quantitation and frequent GC/detector maintenance. With florisil clean up, extracts have lower backgrounds, producing better chromatograms with less interference (Young, 2013) Most stationary phases are based on silica that has been bonded to a specific functional group. Some of these functional groups include hydrocarbon chains of variable length (for reversed phase SPE), quaternary ammonium or amino groups (for anion exchange), and sulfonic acid or carboxyl groups (for cation exchange) (Supelco, 1998).

1.4.2 Gas chromatography-mass spectrometry (GC-MS)

The popularity of GC is based on a favorable combination of very high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity (Steven,

2009). At present, more than 60% of registered pesticides and/or their metabolites are amenable to GC analysis. This has made GC the analytical method of choice for most analytical work involving pesticides (Andrue & Pico, 2004). Fused-silica capillary columns have almost replaced the packed columns, allowing the separation of a large number of analytes with similar physico-chemical characteristics. The selection of columns depends upon the nature of the pesticide(s) to be separated. The identification and separation capabilities of GC can be increased when combined with the confirmation capabilities of the MS. MS is unique in that it not only serves as a qualitative detector, but is equally powerful as a quantitative detector providing detailed structural information. GC-MS systems are also equipped with extensive mass spectral 'libraries' that can be extremely useful for identification and characterization of unknown compounds.

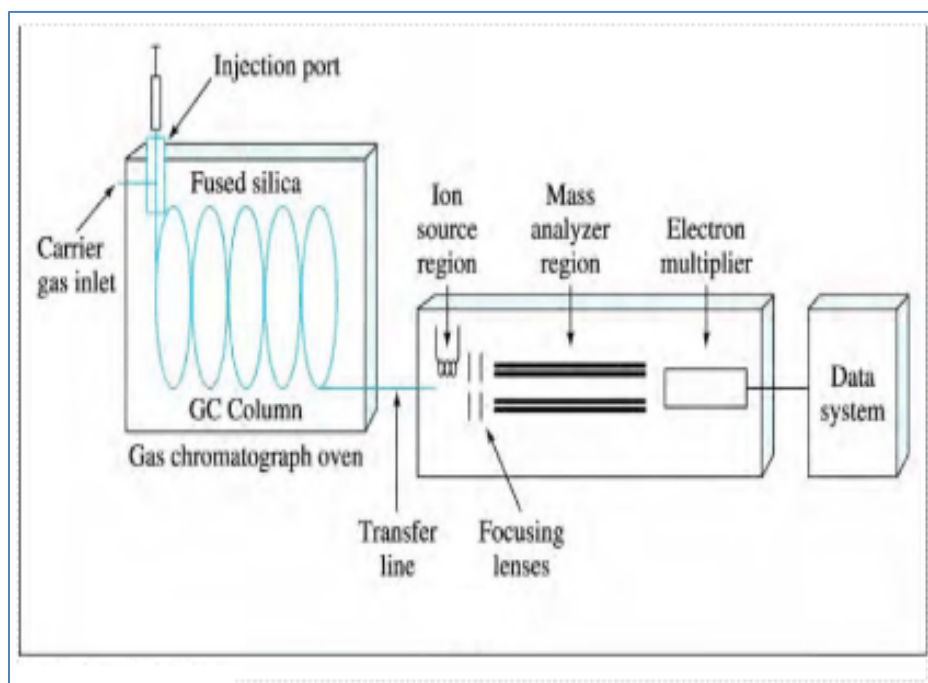


Figure 1.6: Schematic diagram of a GC-MS

GC-MS is usually used in the analysis of mid- polar to non-polar compounds whereas HPLC technique used for polar compounds. Organophosphate and carbamate pesticides are mid polar in nature thus gas chromatography was well suited for their analysis.

1.4.2 Fast GC

According to Sigma Aldrich, (2011) Fast GC is manipulation of a number of features of a column to provide faster analysis times while maintaining resolution. These features include column dimensions, such as the internal diameter (I.D.), length, and film thickness and the type of stationary phase. A fast GC system enables full efficiency and higher levels of production at peak performance times and solves many problems in sample analysis that are problematic with present conventional GC systems (Eiceman *et al.*, 2006). The fast GC column is shorter and more flexible than the traditional GC column, and has a much smaller internal diameter. Conventional GC column internal diameter dimensions are usually at 0.25 mm while fast GC column dimensions are at 0.1 mm for the internal diameter. Similarly, the thickness of the film is greater with traditional GC columns at 0.25 μm vs 0.1 μm with fast GC columns (Agilent Technologies, 2010; Bunn, 2009). Fast GC therefore yields faster analysis times than conventional GC, often three to ten times faster, offering higher throughput and allowing more samples to be analyzed per shift.

1.5 Statement of the problem

Mwea farmers have used organosphosphate and carbamate pesticides in the control and elimination of pests, insects and diseases in their rice farms. This, in turn, has resulted in the entry of the chemical contaminants into the farmlands leading to pollution of the soils, vital water sources and crops. These pesticides are potentially toxic and whenever they are applied; their residues remain on treated environment for a time. Non-point contamination also takes place as the waters from polluted flooded paddies exit the farms as run-off especially during the rainy season contaminating drinking water sources downstream. Carbamate and organophosphate pesticides and their metabolites can be fairly persistent in irrigation schemes, which may pose an ongoing exposure threat to aquatic organisms and birds due to their high toxicity. Furthermore, a big percentage of farmers in the region have limited knowledge in the handling and application of these pesticides, hence most of the time due to lack of protective equipment have prolonged contact with the pesticides (Virginia 1997). Some of these pesticides are systemic in nature and may lead to the death of pollinator insects such as bees and dragon flies. Many of the documented analytical procedures for pesticide residue analysis are complex

and tedious, thus the need of optimization and improvement on existing analytical procedures. As a result of these, it was necessary to carry out the study and determine the pesticide residues in the rice farms. Presently, there is insufficient data on pesticide residues in the Mwea region and the study will go a long way in filling the information gap.

1.6 Justification

This study addresses the need to vigorously monitor the residue levels of agricultural pesticides both in the environment and foodstuff. Presently in Kenya there are no proper mechanisms set out by relevant authorities to monitor the use of pesticides thus the need for continuous screening using high precision instruments such as GC-MS that can positively identify and quantify detected pesticide residues even when at trace levels. Data gathered in this study would help in explaining the changing microenvironment of the Mwea region such the reduction in pollinator insects and reduction of bird population. Myriad health problems that are affecting the farmers and the local population either due to or drinking water from contaminated sources from the region could also be related to the presence of the pesticide residues.

1.7 Hypothesis (Null)

There is no contamination by organophosphate and carbamate pesticides in the rice growing region of Mwea, Kirinyaga County.

1.8 Objectives

1.8.1 General Objective

To determine the levels of selected organophosphate and carbamate pesticide residues in the rice growing region of Mwea, Kirinyaga County.

1.8.2 Specific objectives

1. To develop a simple optimized and validated analytical procedure for the determination of organophosphate and carbamate pesticides.
2. To determine the levels of the detected organophosphate and carbamate pesticide residues in water, soil and rice plant samples.
3. To assess the impact of water pH on degradation of carbamate pesticides.

1.9 Scope and Limitations

The study was a pesticide residue analysis on environmental samples in a rice growing region. Soil, water and rice plant sample were analyzed for carbamate and organophosphate pesticides. Limitations encountered during the study included accessing contact individual to guide us through the vast study area. Challenges in the stabilization of the mass spectrometer during the analysis became a problem due to power fluctuations experienced.

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1 Study area

The study was conducted in the rice fields of Mwea situated on the B6 Embu-Nairobi Highway in Kirinyaga County, approximately 100 kilometres from Nairobi. Located at an elevation of 1,175 meters above sea level the paddy fields lie within coordinates S 0⁰ 42' 0" and E 37⁰ 22' 0". Kirinyaga County is found in central part of Kenya as shown in figure 2.1.

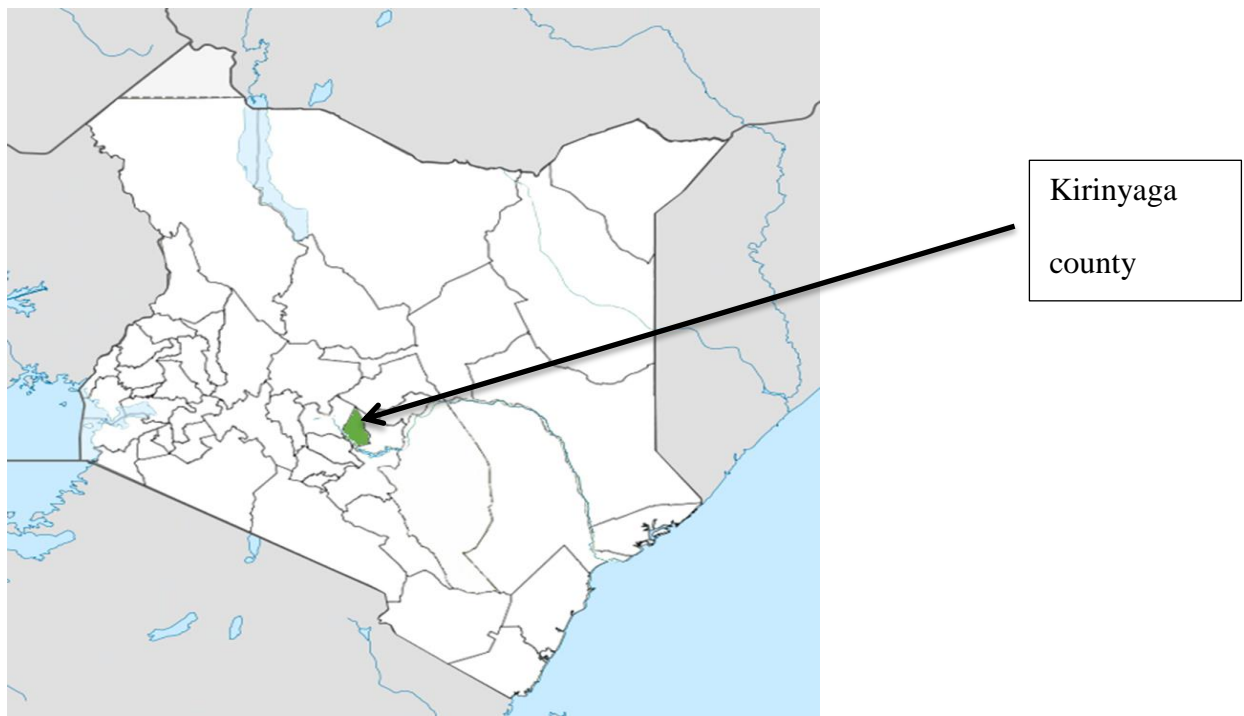


Figure 2.1: Map of Kenya showing Kirinyaga county (Source- Kenya county maps)

The study was carried out between the months of June and August, the year 2013, a season characterized by minimal rainfall. Waters for the irrigation scheme are supplied primarily by Rivers Nyamindi and Thiba. Figure 2.2 shows the location boundaries of the Mwea Sub-County, areas from which samples were collected.

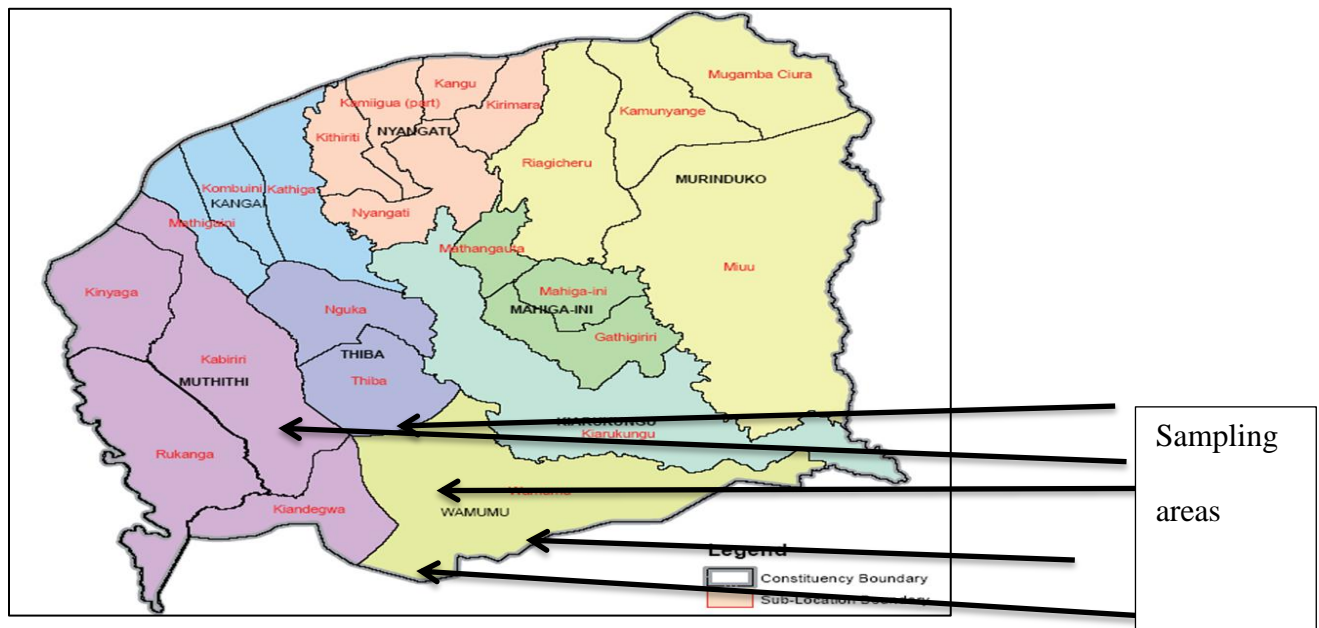


Figure 2.2: Map of Mwea Sub-county showing sampling areas (Source: Sub-location boundaries for Central Kenya Districts, Government of Kenya, 1999.)

Rice farming is the main economic activity in the region it being the most important rice growing region in Kenya, producing over 50% of all rice grown in the country -across 900 hectares. The soil in the area is mainly black cotton in nature. The area has seen the growth of huge milling companies like Mwea Rice Millers and Nice Rice Millers. There are also other small millers that offer employment opportunities. For this reason, many people have migrated to the region from different parts of the country to cultivate and trade in rice.

2.1.1 Sample collection for method validation

Soil samples for method development and validation studies were collected from virgin land within Mwea at an altitude of 1141 meters above sea level within coordinates S 0⁰ 44' 002" and E 037⁰ 18' 080". A hoe was used to dig up the top soil to a depth of 15 cm. Soil collected was black cotton in nature and was transported in clean new paper bags. Paddy water within an experimental rice plot project within Jomo Kenyatta University, Juja main campus farm was

collected and used for water recovery studies. The water was collected in 2.5 L amber colored glass bottles and stored in a fridge at 4⁰C.

2.1.2 Sample collection, transportation and storage

Samples were collected at the expansive rice farms of Mwea sub-county, Kirinyaga along Embu-Nairobi B6 Highway between Mutithi and Ngurubaini Townships. The gradient of the land was taken into consideration during sampling. Soil, water and rice plant samples were collected for analysis. The sampling area was divided into five major sections with further subdivisions within each of these. The sections at the lowest and highest altitudes were Karaba and Mwea- at elevations of 1117 and 1166 metres above sea level respectively. Elevation for Thiba A, Thiba B and Wamumu were at 1150, 1147 and 1134 metres above sea level respectively. Soil samples were collected from ten separate randomly selected spots within a paddy in a section. The collected soil samples from the separate spots within a paddy were mixed together to form a single composite from the section. Further sample reduction on the soil was carried out using the coning and quartering process. Approximately 500g of the soil sample was transported in new paper bags and stored in a fridge at 5⁰C awaiting sample preparation. For water samples, the slow moving (almost stagnant) water within the paddies and from the water channels supplying the rice paddies was collected. The water was transported in 2.5 L amber colored bottles and stored at 4⁰C in a refrigerator in the laboratory prior to analysis. Rice plants within a paddy were uprooted from the ground and the mud on the roots washed off using distilled water, they were wrapped in aluminum foil and transported in a separate cool box. Table 2.1 gives the number of samples collected from each sampling site while Table 2.2 gives a summary of the sampling areas, elevation and the grid references.

Table 2.1: Number of samples collected at each sampling site

Site	Soil	Water	Rice plant
Mwea	2	2	2
Thiba A	2	2	2
Thiba B	2	2	2
Wamumu	2	2	2
Karaba	2	2	2
Water channel 1	–	2	–
Water channel 2	–	2	–
Total	34		

Table 2.2: Sampling sites

Site	Subsection	Altitude (metres above sea level)	Grid references
Mwea	A	1166	S 00 ⁰ 41' 05.9"
	B		E 037 ⁰ 18' 44"
Thiba A	A	1150	S 00 ⁰ 41' 45.9"
	B		E 037 ⁰ 20' 13.5"
Thiba B	A	1147	S 00 ⁰ 41' 44.9"
	B		E 037 ⁰ 20' 13.4"
Wamumu	A	1134	S 00 ⁰ 43' 48.8"
	B		E 037 ⁰ 20' 48.9"
Karaba	A	1117	S 00 ⁰ 43' 55.1"
	B		E 037 ⁰ 23' 05.3"
Mwea (Virgin land)		1141	S 00 ⁰ 44' 00.2" E 037 ⁰ 18' 080"
Water channel 1	Upper section	1166	-
	Lower section	1117	-
Water channel 2	Upper section	1166	-
	Lower section	1134	-

2.2 Experimental

2.2.1 Equipment

- (i) GC-MS, Finnigan GC 8000 series, interfaced with a voyager EI-MS detector (CE Instruments, Milan, Italy) was used for separation, detection and quantification of the samples.
- (ii) Rotavapor R-210 (Buchi Labortechnik AG Postfach, Switzerland) was used for solvent evaporation.
- (iii) Pump-Beckman 110A, used for water sample loading into C18 cartridge
- (iv) pH meter –Hanna Instruments HI 851, for measuring sample pH
- (v) Soxhlet apparatus- Water bath type No. 3456A, for solvent evaporation.

- (vi) Mechanical shaker- Jika Labortechnik KS 250
- (vii) Vacuum concentrator- DNA Mivac 23050-A00, for solvent evaporation

2.2.2 Cleaning of glassware

Glass beakers of 100 ml and 50 ml used in this work were cleaned by soaking in warm water containing a liquid detergent. Rinsing was done in distilled water before drying them in an oven at a temperature of 100⁰C. Volumetric flasks of 100 ml, 50 ml and 10 ml capacity used in standard solution preparation were cleaned by soaking in warm water then rinsing them in distilled water.

2.2.3 Chemicals and reagents

HPLC grade solvents - (methanol, acetone, ethyl acetate and dichloromethane) used for analysis were purchased from Rankem Chemicals, India. The standards carbofuran (CB) (98.5%), 3-hydroxycarbofuran (3-HC) (98%), diazinon (99%) and fenitrothion (99%) were all purchased from Sigma-Aldrich, Germany. C18 SPE cartridges for water extraction were purchased from Sep Pak Waters, while Florosil Bond-Elute cartridges for sample clean-up were purchased from Agilent Technologies, both from USA. Distilled water used was locally prepared at the

laboratory. Stock solutions were prepared by weighing 0.1000 g of each standard and quantitatively transferring it to 100 ml volumetric flask then dissolving in methanol making a concentration of 1000 µg/ml.

2.2.4 Sample preparation

The soil samples were air-dried; ground to powder using a mortar and pestle then sifted through a 2 mm sieve. The prepared soil was stored in a fridge at 5⁰C awaiting analysis. The turbid sampled water was filtered through a Whatman filter paper number 42 with the aid of a suction pump and finally stored in a fridge at 5⁰C. The rice plant samples were air- dried in readiness for extraction.

2.2.5 Optimization of GC-MS conditions

The determination of the pesticides was performed using a Finnigan GC 8000 series, equipped with a Voyager EI-MS detector. The GC-MS conditions were adjusted to obtain the best separation and sensitivity of the analytes by varying injector temperature and adjusting the oven temperature programming. Source and interface temperatures were held constant at 200⁰C and 250⁰C respectively. Helium gas of 99.9999% purity was used as a carrier gas at an inlet pressure of 25 kPa.

2.2.5.1 Column selection

Two columns were compared in the study; an Rtx-5ms of dimension 15m×0.25mm× 0.25µm and a fast GC column, DB-XIb of dimensions 20m×0.18mm×0.18µm. The resolution of peaks, LOD and LOQ achieved by the two columns were compared too. The column with the best resolution and lower LOD and LOQ values was selected for use in the further analysis of the field samples.

2.2.5.2 Injector temperature

Carbamates are fairly thermolabile compounds compared to organophosphate compounds. They can breakdown losing the carbamate moiety at the injection port. It was necessary to determine an optimum injector temperature that would minimize this fragmentation. This was done by injecting 10µg/ml of carbofuran pesticide into the GC at injector temperatures between 100⁰C and 200⁰C. Fifteen injections (15) of the 10µg/ml CB standard were made at each 10⁰C temperature interval rise up to 200⁰C. The highest temperature with the least loss of parent ion from the mass spectra obtained was finally selected as the injector temperature.

2.2.5.3 Determination of limit of detection, limit of quantification and correlation coefficient

The LOD and LOQ were calculated using EPA method 507 (Ripp, 1996) formulae whereby the LOD is three times the standard deviation of 10 fortified one litre distilled water samples with 1 µg/mL of the standards, i.e. $LOD = 3 \times S.D$ of 10 replicates while $LOQ = 10 \times S.D$ of 10 replicates. The linear range of CB, 3-HC, diazinon and fenitrothion was studied by injecting standards of varying concentrations prepared by serial dilution of the 1000 µg/ml stock solutions prepared in methanol. The concentration of the standards prepared varied from 2.5 -15 µg/ml.

2.2.5.4 Repeatability and reproducibility of GC-MS system

The repeatability and reproducibility of the data output from the GC-MS system equipment was investigated. For repeatability test, CB pesticide standard of concentration 5µg/ml was injected seven times within same-day while reproducibility was investigated by injecting the same CB standard seven days later. A comparison on the retention times and peak areas of the eluted analyte was thereafter studied.

2.3 Determination of soil and water pH

Onsite determination of water pH was carried out using a calibrated portable pH meter. Soil pH determination was carried out in the laboratory. The pH of soil samples was determined electrometrically both in water (pH water) and in 0.01 M CaCl₂ (pH CaCl₂) at a (1: 2.5) soil: solution ratio (weight /volume) as outlined by Okalebo *et al.*, (1993). Ten (10) grams of air dried soil samples were added to 25 ml of distilled water and the mixture shaken at 260 reciprocations per minute for 10 minutes and allowed to settle for 30 minutes. The pH of the soil suspension was recorded thereafter, using a pH meter. The pH was measured by carefully immersing the combined electrode into clear supernatant solution. The results were recorded as pH of soil.

2.4 Optimization of Solid Phase Extraction procedure for water samples

The water extraction method was optimized with regard to elution solvent, elution volume and rate of sample loading. The C18 Sep-Pak Plus cartridges were employed in this process. Sample loading was carried out with the aid of a water pump. The SPE procedure consisted of 3 steps; column conditioning, sample loading and sample elution steps.

2.4.1 Selection of SPE elution solvent

Dichloromethane, ethylacetate and methanol were studied as elution solvents for the four pesticides under study, i.e. CB, 3-HC, diazinon and fenitrothion. Five (5) µg/ml of the standards prepared in methanol was spiked separately into 100 ml of test water. All the test samples were prepared in duplicate for each solvent under study.

First, the SPE cartridges were conditioned with 3 ml methanol followed by an equal amount of distilled water. All the spiked water samples, of volume 100 ml each, were then loaded to the conditioned C 18 SPE cartridges.

The loaded cartridges were eluted with two aliquots of 5 ml each of ethyl acetate, DCM and methanol. The extracting solvent was then reduced using a vacuum concentrator to 1 ml for GC-MS analysis. Finally, 1 µl of each extract was injected into the GC-MS and the peak area for

each solvent extract was used to determine their percentage recovery. The solvent which yielded the highest percentage recovery was used in the analysis.



Figure 2.3: Set up showing loading water sample into SPE cartridge

2.4.2 Selection of SPE elution volume

Studies were conducted to determine the optimum elution volume for the analytes retained in the adsorbing material. Spiked water samples of 100ml volume, were loaded into the SPE cartridges then eluted with increasing volumes of methanol. The first set was eluted with 2 aliquots of 1 ml each, the second, third and fourth sets were eluted each with 2 aliquots of 2 ml, 3 ml and 5 ml of methanol respectively. This procedure was carried out separately for the four standards under study. The volumes were then reduced with a vacuum concentrator to 1 ml. One microliter of the extract was injected thrice into the GC-MS and the peak area was used to compute the percentage recovery for each set. The least volume that gave highest recovery was used as the elution volume in the subsequent elution steps.

2.4.3 Determination of sample breakthrough volume

This was determined by spiking CB and diazinon standards into the test water at two concentration levels (5 µg/ml and 10 µg/ml). Water volumes under study were 50 ml, 100 ml, 250 ml, 500 ml and 1000 ml. Each sample was loaded into a conditioned SPE cartridge for extraction and then eluted with two aliquots of 3 ml methanol. The extracts were then concentrated to 1 ml for analysis. For each extract, an aliquot of 1.0µl was injected into the GC-MS thrice and the average peak area used to determine the recoveries from the calibration equation and hence the breakthrough volume of the SPE columns.

2.4.4 Determination of flow rate

Bidlingmeyer (1984) reported that recovery is dependent on flow rate through the SPE device, recovery is reduced at at higher flow rates. Different water sample loading flow rates were studied. The flow rate was varied from 1 to 10 ml/min in steps of 1, 2, 4, 6 and 10 ml/min. Test water was spiked separately with 5 µg /ml of CB, 3-HC, diazinon and fenitrothion then loaded to conditioned SPE cartridges at the varied flow rates. Elution was carried out and the volume reduced by vacuum concentrator to 1 ml then injected thrice to the GC-MS. The average peak area was used to calculate percentage recovery from the calibration curve.

2.4.5 Water recovery studies at optimum conditions

Using the optimum conditions, recovery studies for the four pesticides in 250 ml and 350 ml of test water was carried out at two concentration levels (5 µg/ml and 10 µg/ml). At each spiking level, the studies were done in duplicate for each pesticide. The pesticide spiked water was extracted following the optimum conditions earlier obtained and the resulting volume concentrated to 1 ml. The extracts were then injected into the GC-MS and the average peak area was used to compute the recovery.

2.4.6 Liquid-liquid extraction

LLE was carried out in order to find out if there would be any significant difference in terms of the recoveries between it and the SPE method of extraction. Dichloromethane and ethylacetate were employed as solvent of choice since they are immiscible with water. Three hundred and fifty milliliters (350 ml) of test water in a separating funnel was spiked with 5 µg/ml of the standards. One hundred milliliters (100 ml) of DCM and ethyl acetate were used in the extracting process. The mixture was shaken, after which partitioning took place and the organic phase collected while the aqueous discarded. Solvent evaporation was carried out using a vacuum evaporator to 1 ml. The extract was then injected into the GC-MS thrice and the average peak area used to compute the recovery.

2.5 Soil analysis

Soil samples were air-dried thereafter ground using pestle and mortar. Sifting was carried out using a 2 mm sieve. Twenty grams (20 g) of the soil was weighed followed by addition of 15 g of anhydrous sodium sulphate; the mixture was homogenized to ensure maximum removal of any residual moisture. The soil sample was spiked with 5µg/ml of the CB and diazinon (standing in for carbamates and organophosphate standards respectively). After spiking, a residence time of 12 hours was allowed for the pesticide spike to equilibrate with the soil sample. Extraction of the spiked samples was carried out using both the soxhlet and mechanical shaker method of extraction.

In the soxhlet method of extraction, 20 g of the spiked soil was placed into a pre-cleaned thimble (using dichloromethane) and covered by cotton wool. Acetone, DCM and ethyl acetate of volume 200 ml for each were used in the extraction process at duration of 6 hours each. The extracts were reduced using vacuum concentrator to 1ml. The 1 ml extract was filtered using a veritpure- 0.2 µm nylon syringe filter. Thereafter the extract was loaded onto Florosil-Bond-Elut column (magnesium silicate) conditioned with methanol for sample clean-up. Ten milliliters of methanol was used for analyte elution. The extracts were reduced using a rotary evaporator to 1ml and then 1µl of extract injected thrice into the GC-MS.

In the mechanical shaker method of extraction, 20 g of spiked soil samples were placed in 250 ml conical flasks. Two hundred milliliters (200) of acetone, DCM and ethyl acetate were used as the extraction solvent. The conical flasks were carefully covered with foil paper, the shaker operated at 200 revolutions per minute and extraction duration set for 6 hours. The supernatant was collected and filtered. Solvent evaporation was carried out using a rotary evaporator to 1 ml further filtration of the extract was carried out using veritpure- 0.2 μm syringe filters. Cleaning of the extract was carried out using a conditioned Florosil -Bond elut cartridge (magnesium silicate). The samples were run in a GC-MS and the recovery results compared with the samples extracted using soxhlet method.

2.5.1 Soil clean-up elution volume

A test to select the optimum volume to elute most analyte during soil sample cleanup was carried out. Sample extract of volume 1 ml was loaded into a conditioned florosil cartridge. Methanol of volume 3 ml, 6 ml and 9 ml was then used for sample elution and the recoveries calculated.

2.6 Rice plant analysis

The rice plant samples were air-dried, cut to small pieces of approximately 3 mm and finally ground using pestle and mortar. Twenty (20) grams of the samples were spiked separately with 5 $\mu\text{g}/\text{ml}$ of the four standards. The soxhlet method was used as the method of extraction. Acetone and DCM were studied as possible solvents of extraction. Solvent evaporation was carried out using a rotary evaporator. A plug of 3 g of activated charcoal was placed on top of the florosil Bond elut cartridge during sample clean-up to decolorize the sample. The 1 ml extract was filtered using a veritpure- 0.2 μm nylon filter. Seven milliliters of methanol was used for sample elution. The extracts were reduced using a vacuum concentrator to 1ml then injected in the GC-MS. The recoveries were calculated from the peak areas obtained.

2.7 Effect of pH on CB and 3-HC availability

The pH of 250 ml distilled water was adjusted by adding 0.01M hydrochloric acid and 0.01M sodium hydroxide drop wise to induce a drop or an increase of its pH respectively. A pH meter

was used to monitor the induced pH change. Water samples with pH range of 2 to 12 were prepared in duplicate at each 2 pH unit rise. Five (5) µg/ml of CB and 3-HC standard was spiked into the waters at the different set pH values. Two hours was allowed for the spiked standard to equilibrate with the waters. Spiked pesticides were extracted and recovery studies carried out.

2.8 Data Analysis and interpretation

Data was analysed using MS Excel and Genstat software version 14 for analysis of variance (ANOVA). The 95% confidence interval was used as a criterion of statistical significance

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 Optimization of GC-MS conditions

3.1.1 Oven temperature programming

By varying the initial temperature and temperature gradient it was found out that the optimum GC program for the analysis of CB, 3-HC, diazinon and fenitrothion to be: initial temperature of 60°C held for 2 minutes then the temperature ramped at a rate of 15°C/min to 240°C which was maintained for 10 minutes. The total run time was approximately 24 minutes.

3.1.2 Injector temperature

Loss of the molecular ion of CB was observed at temperatures of 160°C and above. Tatsuhiko and Nobuko (2003), using a GC system equipped with a temperature programmable inlet on-column injection system observed that CB was getting transformed to carbofuranphenol at 140°C. In this present study, an injector temperature of 180°C was selected at which 10% of the runs experienced loss of the molecular ion. Tatsuhiko and Nobuko (2003), using the conventional split less injection mode had 3% of CB runs experiencing loss of the molecular ion and being converted to carbofuranphenol at 200°C injection port temperature. Figure 3.1 shows the mass spectrum of CB, with base peak at m/z 164 and parent ion m/z 221. Loss of parent ion on CB and 3-HC was observed at temperatures above 180°C as shown in appendices 1 and 2 respectively. The difference between Figure 3.1 and appendix 1 is that in figure 3.1 the parent ion of the compound CB (m/z 221) is still intact while in appendix 1, we observe the loss of the parent ion. The base peak for 3-HC was m/z 137 and its molecular ion m/z 237 this is clearly shown in appendix 3. Appendix 2 shows the mass spectrum of 3-HC with the loss of the parent ion (m/z 237). Fenitrothion and diazinon were stable under the set injector temperatures and no loss of their parent ion was observed. The mass spectra of these two pesticides were intact under the set injector temperature and their parent ion clearly visible as shown in Figures 3.12 and 3.13 respectively.

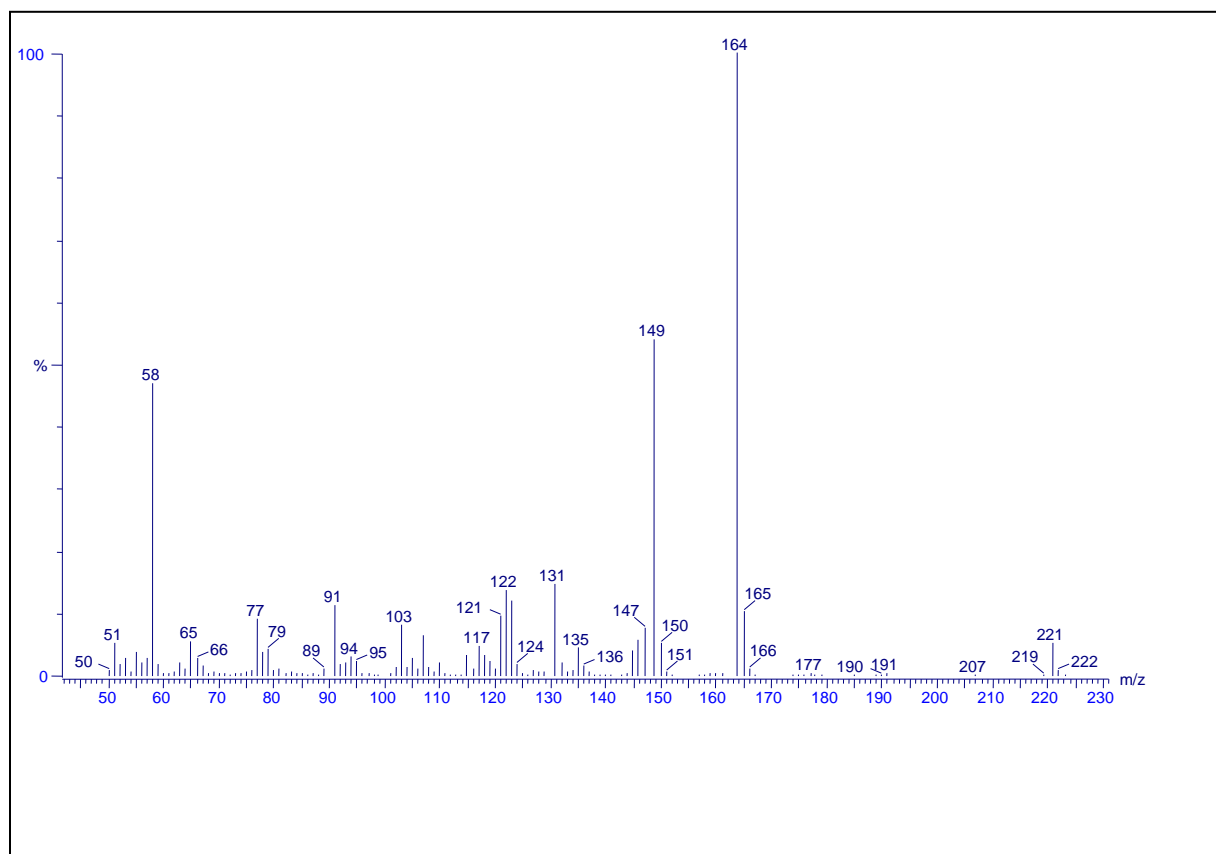
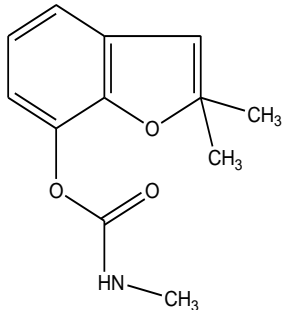
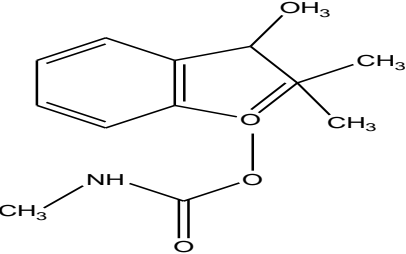
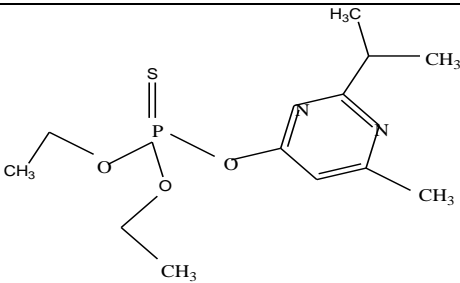
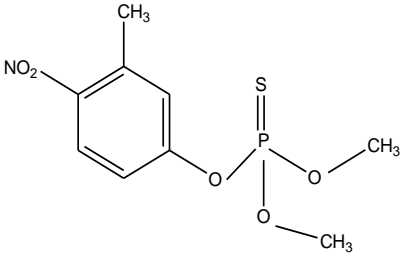


Figure 3.1: Mass spectrum of carbofuran showing the parent ion (m/z 221) intact.

Table 3.1: Main mass fragments of CB, 3-HC, diazinon and fenitrothion

Compound	Structure	MW(g/mol)	m/z (some mass fragments)
Carbofuran		221	164 [M-CH ₃ -NH-CO] ⁺ 149 [M-(CH ₃) ₂ -NH-CO] ⁺ 132 [M-(CH ₃) ₂ -NH-CO-OH] ⁺
3-hydroxy carbofuran		237	137 [M-(CH ₂) ₃ -NH-CO-OH] ⁺ 147 [M-CH ₃ -NH-CO-(OH) ₂] ⁺ 163 [M-CH ₃ -NH-CO-OH] ⁺ 180 [M-CH ₃ -NH-CO] ⁺
Diazinon		304	137 [M-(C ₂ H ₄ O) ₂ PS] ⁺ 152 [M-C ₃ H ₅ O ₂ PS] ⁺ 248 [M-C ₄ H ₈] ⁺ 276 [M-C ₂ H ₆] ⁺
Fenitrothion		277	108 [M-(CH ₂ O) ₂ PS-NO ₂] ⁺ 125 [M] ⁺ 261 [M-CH ₃] ⁺

3.1.3 Column selection

The major difference between the conventional column and the fast GC column in this study was the internal bore diameter, film thickness and internal coating material. The fast GC column dimensions were internal bore and film thickness of 0.18mm×0.18µm respectively while the conventional column was 0.25 mm×0.25 µm. According to Sigma Aldrich (2011), even with the change of one feature of a column, better performance is expected by a fast GC column. Hubschman (2009) found that reducing the internal bore improves the resolving power of the column thus achieving higher chromatographic resolution. It also reduces analysis time. He also found that thin films of ca. 0.1 µm are very effective with GC/MS. Thin films give narrow rapid peaks and can be used in higher temperature ranges of about 320-340⁰C (Hubschman, 2009) without significant column bleed. This is clearly shown in Figure 3.2 TIC where there is proper resolution of the analyte peaks as contrasted with Figure 3.3 which illustrates the TIC obtained by the conventional column. The runs were carried out under same chromatographic conditions. Furthermore, the fast GC column was able to resolve clearly the phenol transformation products of both CB and 3-HC a feat not clearly resolved by the Rtx-5ms column. Facchetti (2005) used a Fast GC column, Trace TR-5MS in separation of 16 PAHs. He was able to reduce the analysis time from 18 minutes obtained by a similar Trace TR-5MS conventional capillary column to 10 minutes while at the same time maintaining good resolution. This test was done under same chromatographic conditions for the two columns too. Peak 1 in Figure 3.2 represents carbofuranphenol while peaks 2, 3 and 4 represent 3-hydroxycarbofuran phenol, 3-HC and CB respectively. No transformation product was observed in Figure 3.3, peak at 1 and 2 represent 3-HC and CB respectively. Hence, the fast GC-Db-XIb column was selected and employed in all the GC-MS analyses work.

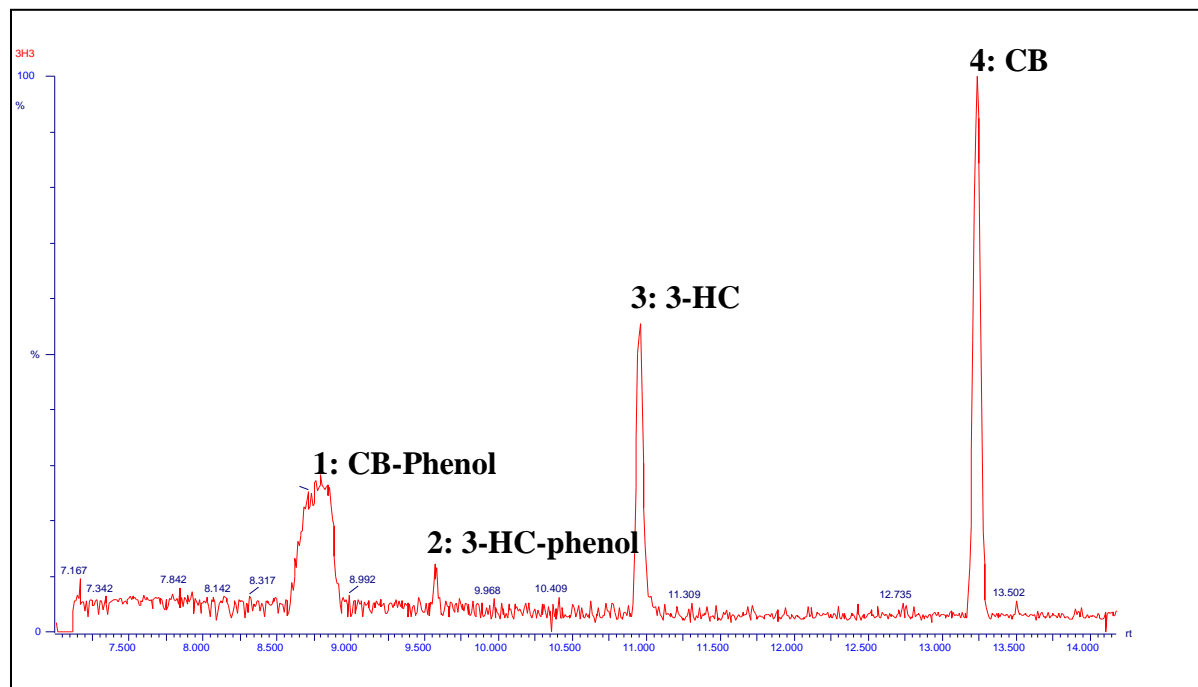


Figure 3.2: TIC resulting from the fast GC column, DB-XLB

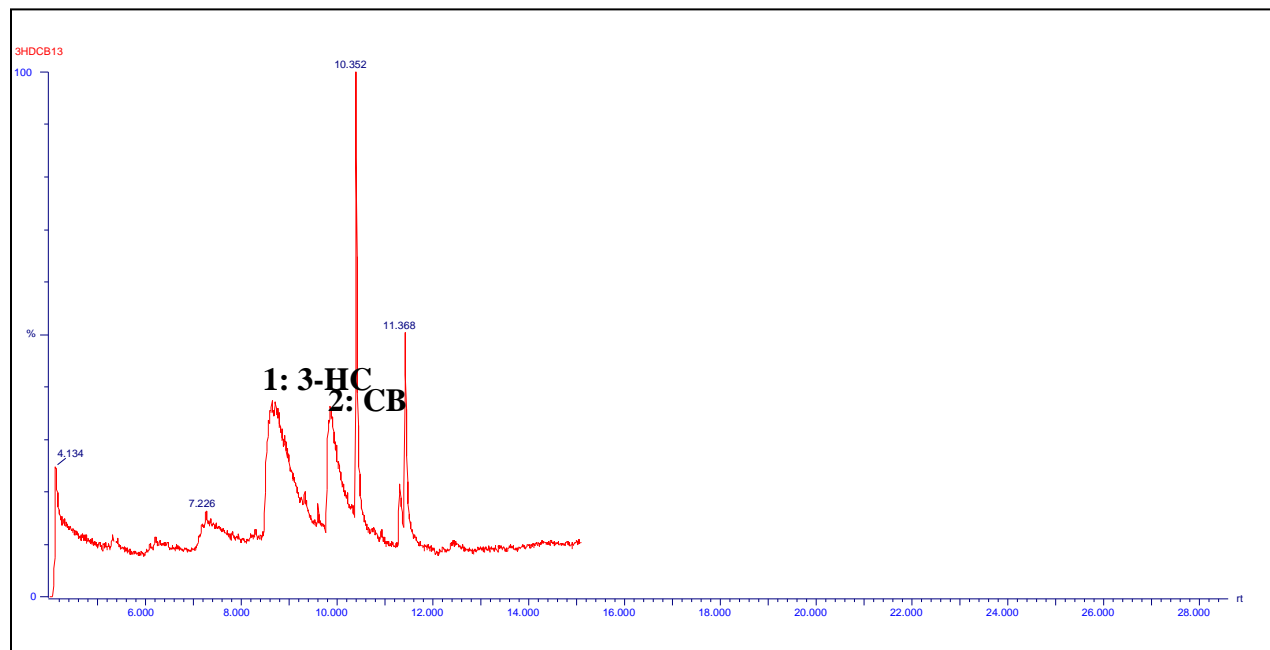


Figure 3.3: TIC resulting from the conventional column, Rtx-5MS

3.1.4 Calibration curves, Limit of detection (LOD) and Limit of quantification (LOQ)

The correlation coefficient (R^2) value of CB and 3-HC was 0.997 and 0.994 and 0.997 and 0.995 for diazinon and fenitrothion respectively an indication that the calibrations were linear in the concentration range. This is clearly shown in figures 3.4 and 3.5.

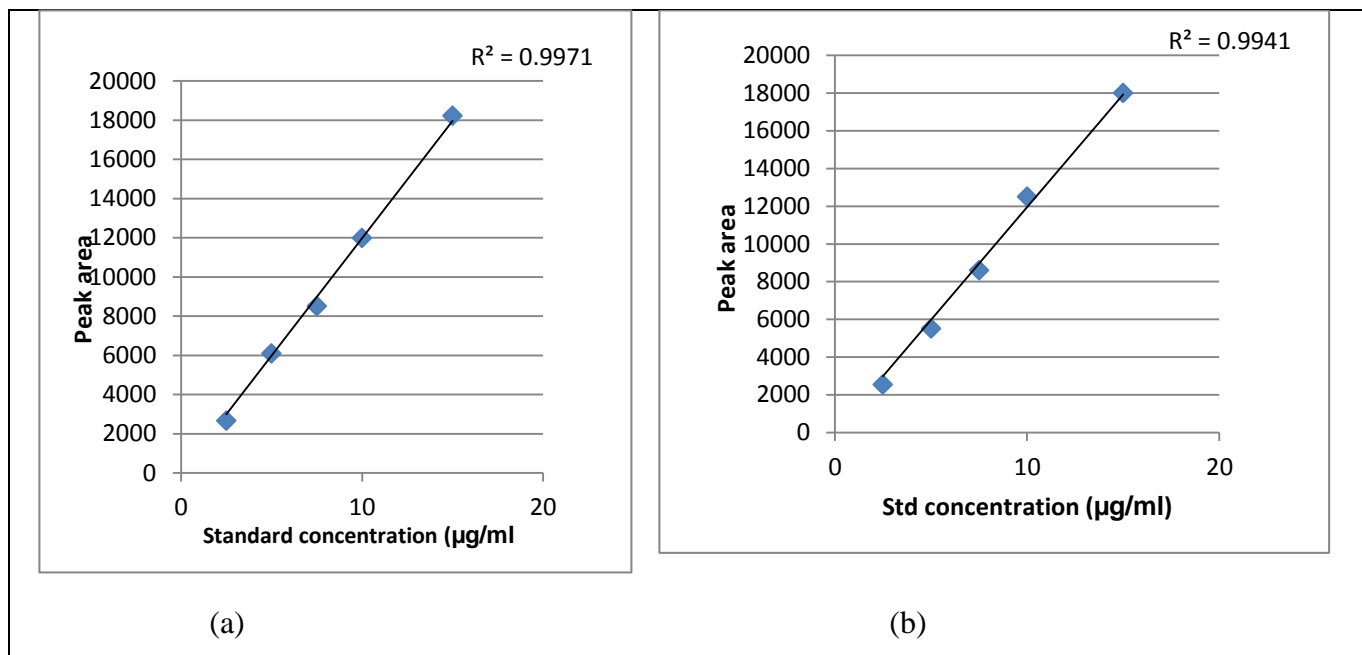


Figure 3.4: Plot of peak area vs concentration of (a) carbofuran and (b) 3-HC



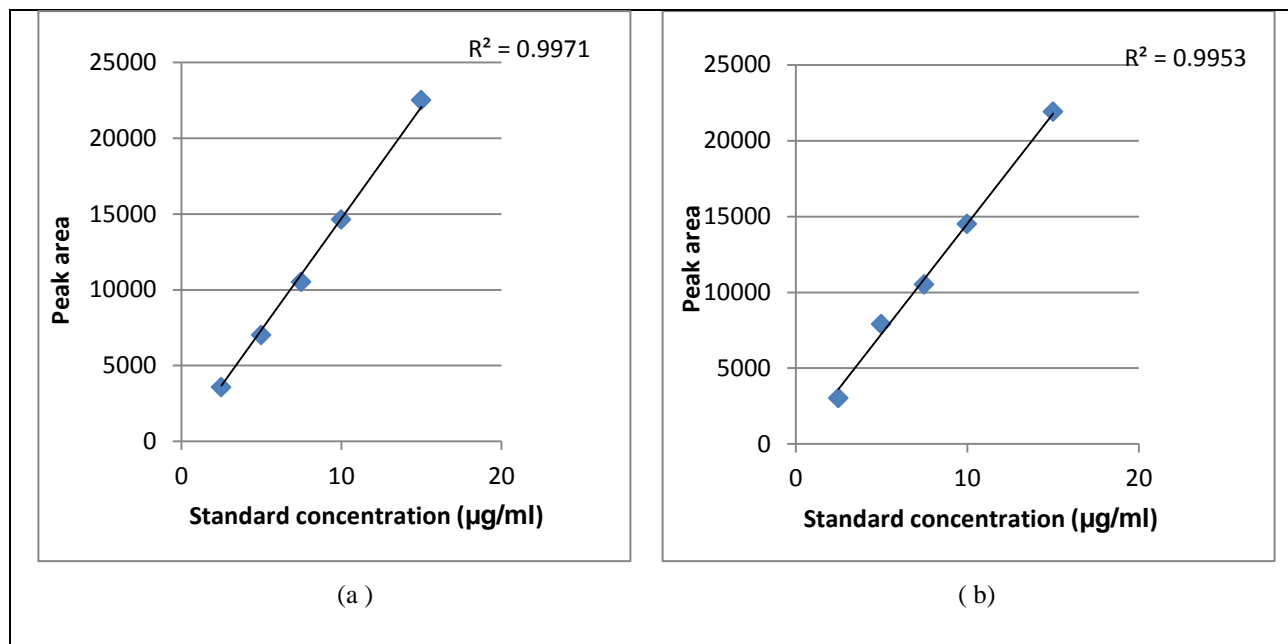


Figure 3.5: Plot of peak area vs concentration of (a) diazinon and (b) fenitrothion

The method used in determination of LOD and LOQ is based on the principle that since the peak area of the blank is zero, it is not possible to calculate the LOD using the blank; and hence the standard deviation of the concentrations of fortified samples is assumed to be equal to the standard deviation of the blank. A summary of the results is provided in Table 3.1. Lower LODs and LOQs values were obtained using the DB-X1b further displaying its superior performance characteristics.

Table 3.1: LOD and LOQ values as yielded by the two columns

Standard	column	LOD ($\mu\text{g/l}$)	LOQ ($\mu\text{g/l}$)
CB	Db-Xlb	0.38	1.20
	Rtx-5MS	0.58	1.93
Diazinon	Db-Xlb	0.11	0.43
	Rtx-5MS	0.23	0.76
3-HC	Db-Xlb	0.54	1.74
	Rtx-5MS	0.78	2.60
Fenitrothion	Db-Xlb	0.17	0.56
	Rtx-5MS	0.35	1.16

3.1.5 Repeatability and reproducibility data of the GC-MS system

Using a manual injection system, the GC-MS system yielded repeatable and reproducible results that were satisfactory as indicated in Table 6. Khin and Somporn (2007) carried out similar test with a HPLC system equipped with an auto sampler to determine levels of carbofuran and carboxin in cabbages, thus their system yielded better reproducibility values than the present study as indicated in Table 3.2.

Table 3.2: Repeatability and reproducibility studies

	This study (manual injection)		Khin and Somporn (2007) (automatic injection)	
	Same day %RSD (n=7)	Intra-week %RSD (n=7)	Same day %RSD (n=7)	Intra-week %RSD (n=7)
Retention time	3.23	3.43	0.61	2.7
Peak area	3.80	4.21	0.22	0.67

3.2 Determination of soil and water pH

In the assessment of the aquatic environments, physical characteristics of a water body need to be investigated and evaluated in order to obtain meaningful interpretation of the results (Bartram & Balance, 1996). Table 3.3 shows the distribution of pH in irrigation paddies on the fourteen sampling points. The observed mean water pH values in the sampling points within the paddies ranged between 5.56– 6.42. The water channels supplying the paddies had mean pH ranging from 6.45- 6.88. The water within the paddies had slightly lower pH than the water from the channels.

Table 3.3: Onsite water and soil pH values

Section	Subsection	Water	Soil
Mwea	A	6.13±0.26	5.63±0.15
	B	6.42±0.13	5.48±0.06
Thiba A	A	5.92±0.07	5.41±0.05
	B	5.92±0.045	5.43±0.02
Thiba B	A	6.16±0.25	5.35±0.01
	B	5.96±0.08	5.43±0.02
Wamumu	A	5.68±0.01	5.29±0.07
	B	5.76±0.08	5.44±0.04
Karaba	A	5.56±0.07	5.42±0.09
	B	5.62±0.06	5.43±0.02
Water Channel 1	Upper	6.84±0.05	-
	Lower	6.45±0.06	-
Water Channel 2	Upper	6.88±0.01	-
	Lower	6.76±0.12	-

Mean ± SD (n=3)

Mwea in Kirinyaga County same as Kano plains in Kisumu County has planosols and vertisols type of soil which occur on very gently undulating to flat topography. These soils are dark coloured and strongly cracking (Gachene & Kimaru, 2003). The mean soil pH varied from 5.29-5.63 which is very strongly acidic according Soil Survey Manual (1993). Kihoro *et al.*, (2013) while creating a suitability map for rice growing in Mwea found the soil pH values varying from 5.6 to 7.3. Studies carried by Chemining'wa *et al.*, (2013) on Kirinyaga South soils found the pH values ranging from 4.3-5.6. Rice growing soils in Bangladesh, one of the world's leading producers of rice were found to range from 5.5-8.4 (ICID, 2012). According to Elisa *et al.*, (2011), the critical pH for rice growth is 6. The soil pH values of this study did show that the Mwea soils to have slightly lower pH from the critical rice soil pH. In comparison with the Bangladesh soils too, the soils tended to have slightly lower pH. Generally on each section and

paddy, soil pH was found to relatively lower than the water pH however, there was no significant difference ($p < 0.05$) between soil and water pH.

3.3 Optimization of the SPE method for water extraction

3.3.1 Elution solvent

The selection of the solvent for extracting the pesticides was based on several criteria. The major consideration for a solvent choice depends on polarity of the pesticides. The polarity of the solvent must be sufficient to extract most pesticides from the matrix samples. The solvent must also extract all pesticides and be rapidly evaporated in large volume. The solvents investigated in this study were dichloromethane, ethyl acetate and methanol. The three solvents have polarity indices of 3.1, 4.4 and 5.1 respectively (Snyder, 2012). The test water used in the recovery studies had pH values between 6.4-6.8. Table 3.4 shows that methanol yielded the highest recovery for all the four standards under study with a percentage recovery of 80-84%. Ethyl acetate yielded equally high recoveries for diazinon and fenitrothion which was at 78-80%. Methanol was used as elution solvent in all the elution steps.

Table 3.4: Mean percentage recovery of the pesticides eluted with solvents of different polarities

Solvent	Carbofuran	3-HC	Diazinon	Fenitrothion
Methanol	84.13±1.98	80.54±3.25	82.48±2.57	84.28±3.21
DCM	73.86±4.67	74.36±3.59	76.45±3.68	73.25±2.09
Ethyl acetate	50.73±3.07	43.25±2.58	80.26±4.01	78.77±3.03

Mean ± Sd (n=3)

3.3.2 Elution volume

Elution volume was varied to obtain the optimum volume for eluting most of the adsorbed analyte. The C18-SPE cartridge was first conditioned by 3 ml methanol followed by equal amount of distilled water. Methanol was used as elution solvent for the C18-SPE cartridge. The elution volumes studied were in the range of 2-10 ml. Figure 3.6 shows that recoveries increased

with increasing elution volume. The optimum volume that gave highest recoveries was 6 ml of methanol i.e. two aliquots of 3 ml methanol.

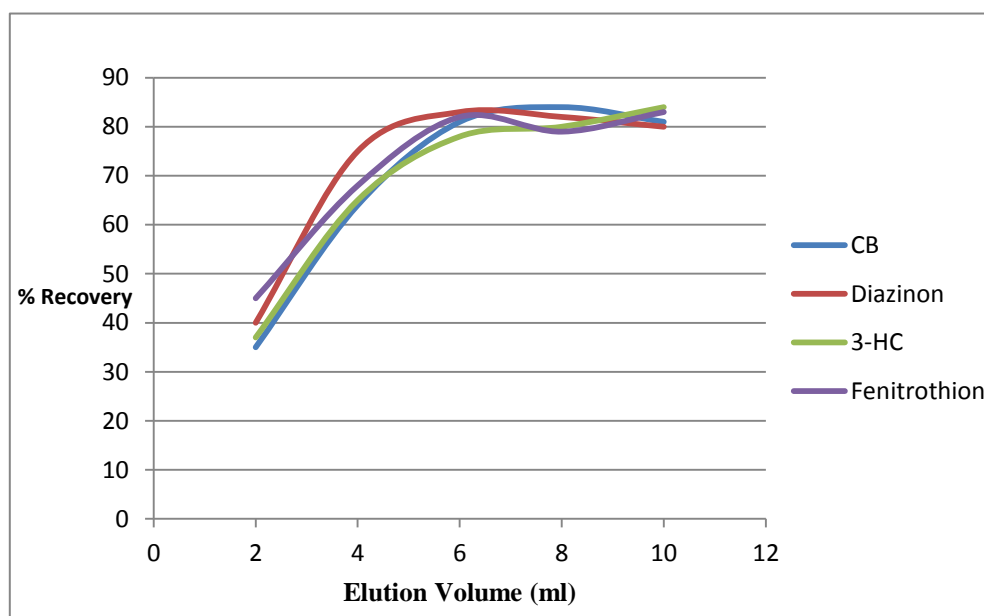


Figure 3.6: Recoveries of the pesticides using varying volumes of the eluting solvent (methanol)

3.3.3 Determination of sample breakthrough volume

When dealing with large volume samples, the breakthrough volume of certain analytes may be exceeded. Sample breakthrough is a function of the strength of the interaction between the analyte and sorbent, the sample volume and the mass of sorbent (Wells, 2000). The extraction of the pesticides was carried out using tC18 cartridges packed with 360 mg of sorbent material. The recovery data on the breakthrough volume test is represented in the Table 3.5.

Table 3.5: Breakthrough volumes

Volume (ml)	CB		Diazinon	
	5µg/ml	10µg/ml	5µg/ml	10µg/ml
50	83.68±2.36	82.96±1.99	79.91±2.05	81.69±3.47
100	80.37±3.25	83.38±2.89	79.81±0.98	78.41±2.56
250	79.31±0.09	79.21±2.41	76.36±2.33	77.96±1.11
500	75.39±4.36	59.0±3.66	73.52±4.01	54.21±2.98
1000	38.26±4.21	45.54±3.65	29.63±3.74	35.23±0.21

Mean ±Sd (n=3)

At 5µg/ml spiking level, the recoveries for both CB and diazinon from 50-500 ml solutions ranged from 73 – 83%. A drop in the recoveries was observed at volumes between 500-1000 ml for CB and diazinon respectively. The lowest observed recovery of the analytes was at 1000ml and was 38% and 29% for CB and diazinon respectively. The breakthrough volume was about 400 ml at 5µg/ml spiking level. At the 10µg/ml spiking level, the observed breakthrough volume was about 250 ml. The highest recovery of the analytes at this spiking level was observed at volumes between 50-250 ml which was 79-82% and 77-81% for CB and diazinon respectively. The lowest observed recovery was 35%.

3.3.4 Sample loading flow rate

Mayer and Poole (1994) found that the recovery of analytes by SPE shows significant flow-rate dependence. Sample loading can be time consuming when working with large volumes of sample thus an optimum rate had to be established. A flow rate that is fast enough but that also ensures that all analyte of interest is retained by the packing material material. Lower recoveries of 40-60% were obtained at faster flow rates of 5-8 ml/min in all standards as shown in Figure 3.7. Recoveries of 81-85% were obtained at the slower loading rates of 1-4 ml/min. The optimum flow rate for sample loading was found to be 4ml/min.

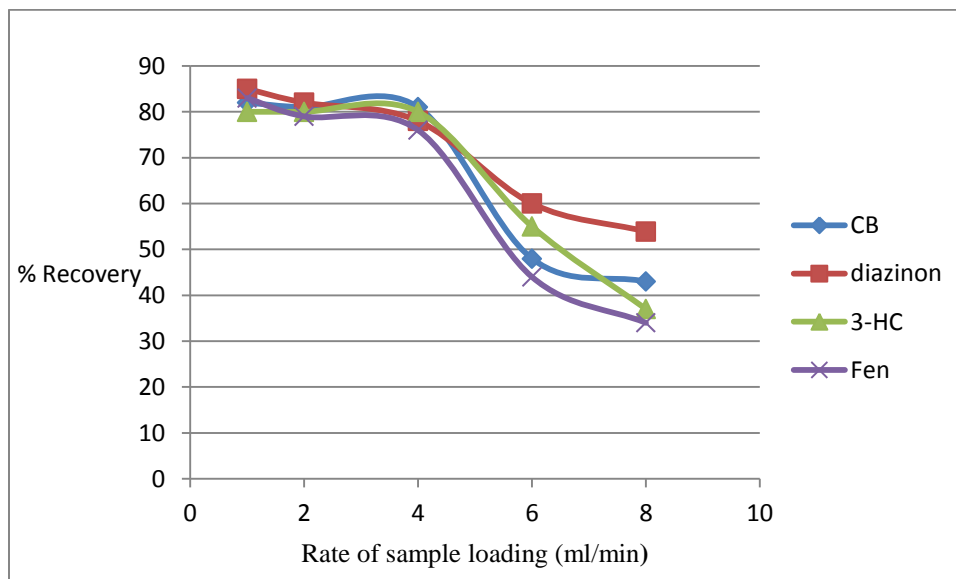


Figure 3.7: Recoveries obtained at different water sampling flow rates

3.3.5 Recoveries at optimum conditions

SPE recoveries were determined using the optimum conditions of elution solvent, elution volume, break through volume and flow rate of sample loading obtained at two spiking levels of 5µg/ml and 10µg/ml. Recoveries of 80-85% and 78-82% were obtained at the 5µg/ml and 10µg/ml spiking level respectively. as shown in Table 3.6.

Table 3.6: Recoveries obtained at optimum conditions

Spike	CB	3-HC	Diazinon	Fenitrothion
5µg/ml	82.13±1.98	80.93±1.96	85.18±2.68	85.11±2.58
10µg/ml	81.52±2.12	78.37±2.01	79.35±1.48	81.99±2.58

Mean ± Sd (n=3)

A study by Young *et al.*, (2001) on C18 SPE recoveries on carbamate pesticides (aldicarb, carbaryl and carbofuran) from water yielded recoveries from 80-98%. Otieno *et al.*, (2010)

obtained mean recoveries of 88-90% and 75-78% on CB and 3-HC from soil while Chowudhry *et al.*, (2012) were able to obtain recoveries of 81% diazinon from water.

3.3.6 Liquid-liquid extraction in water extraction

A comparison was made between the LLE and SPE method of extraction. This was to evaluate which of these two extraction procedures yielded better recoveries. Table 3.7 shows that with DCM solvent, recoveries of 74-78% were obtained as compared to ethyl acetate 50-76%. It was however noted that higher recoveries of 83-84% were realized in the SPE method (using methanol as elution solvent). DCM in LLE yielded recoveries of 74-78% which is comparable to when it was employed in the SPE method (recoveries of 73-76% were obtained). In the long run, SPE process was settled for as the method of choice.

Table 3.7: Recoveries from solvents of different polarities in the LLE method of extraction

Solvent	CB	3-HC	Diazinon	Fenitrothion
DCM	78.32±4.33	78.05±3.35	77.02±5.49	74.54±5.24
Ethyl acetate	54.12±3.54	50.24±3.69	75.55±4.77	76.58±4.21

Mean ± Sd (n=3)

The SPE method consumed lesser amount of solvent. There was minimal use of glassware in the SPE method minimizing probability of contamination and cutting down on time used to clean such glassware. Furthermore better precision values were observed in the SPE method as compared to the LLE method of extraction. SPE method had precision values (% RSD) values ranging from 2.43- 3.14% while LLE precision values ranged from of 4.29-7.30% (n=3). Methanol was not tested in the LLE method of extraction since it is miscible with water thus partitioning process would have been impractical.

3.4 Soil analysis

3.4.1 Extraction solvent

Soxhlet method yielded averagely higher recoveries for all solvents under study as compared to the mechanical shaker method of extraction in soil extraction method. There was significant difference ($p < 0.05$) between the two methods. Acetone yielded highest recoveries in the soxhlet method at 80% for both CB and diazinon. The highest recoveries in the mechanical shaker method were 64-65% as shown in Table 3.8.

Table 3.8: Comparison between mechanical and soxhlet method of extraction

Solvent	Soxhlet		Mechanical shaker	
	CB	Diazinon	CB	Diazinon
Acetone	80.86±2.91	80.11±1.89	64.48±3.22	65.52±3.69
DCM	76.89±1.17	79.04±2.94	58.66±4.25	63.93±3.47
Ethyl acetate	45.87±1.36	75.55±2.22	38.64±4.54	60.09±3.40

Mean±Sd (n=3)

Ethyl acetate gave the lowest recoveries of 38% in the extraction of CB in mechanical method. Generally, better precision values of 1.52-3.74% were observed in soxhlet method as compared to 4.99-11.74% in the mechanical shaker method. Soxhlet was adopted as the method of extraction in soil analyses with acetone as the solvent of choice on pesticide extraction from soil.

3.4.2 Elution volume

Methanol was used as the elution solvent during sample cleanup step. Florosil-Bond elut (magnesium silicate) cartridges were employed in the process. An optimum elution volume for the sample cleanup process had to be determined. With increasing volume, higher recoveries were obtained. The optimum elution volume was found to be 7 ml as shown in Figure 3.8.

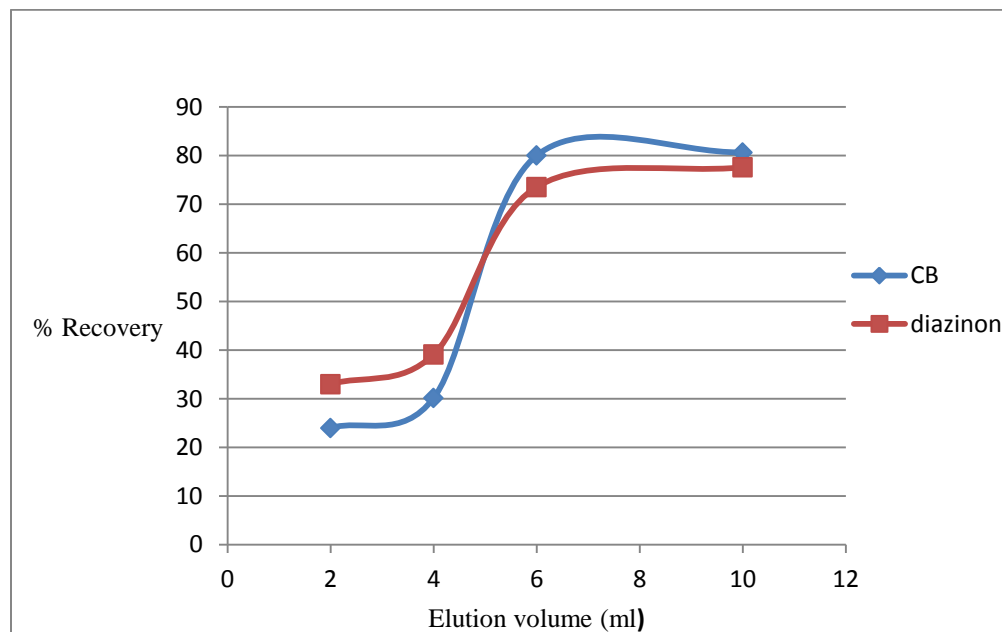


Figure 3.8: Optimum elution volume for florisil clean up in soil analysis

3.5 Rice plant analysis

3.5.1 Extraction solvent

Sohxlet method of extraction was employed in pesticide extraction from vegetation sample. Acetone and DCM were studied as possible solvents for extraction in the extraction of the spiked rice plant. According to Table 3.9, acetone gave higher recoveries of 75-78% while DCM had slightly lower recoveries of 70-73%. Acetone was selected as the solvent of choice. Seven milliliters of methanol was used as the elution solvent in sample cleanup in the sample clean up step.

Table 3.9: Recoveries from solvents of different polarities in the vegetation analysis

Solvent	CB	3-HC	diazinon	Fenitrothion
Acetone	76.21±3.25	75.54±2.58	78.89±2.15	76.77±3.61
DCM	70.35±2.89	73.55±2.33	72.01±1.36	72.73±3.44

Mean±sd (n=3)

3.6 Effect of pH on CB and 3-HC availability

Chemical parameters such as pH enhance or depress the general physico-chemical conditions prevailing in a water system (Adefemi *et al.*, 2007). The pH of an aquatic system is an important indicator of the water quality and extent of pollution in an area. In this study, CB and 3-HC standards were spiked into distilled water at different pH levels. Figure 3.9 shows that pH is a major parameter that influences the availability of a pesticide in the environment.

For CB, at pH levels of 2 -6 recoveries of 79-84% were realized. At pH 7, the spiked water was at neutral and a recovery of 75 % was observed. Above pH 7 a sharp decline in recoveries was observed. The lowest recoveries of 22% were observed at pH 12

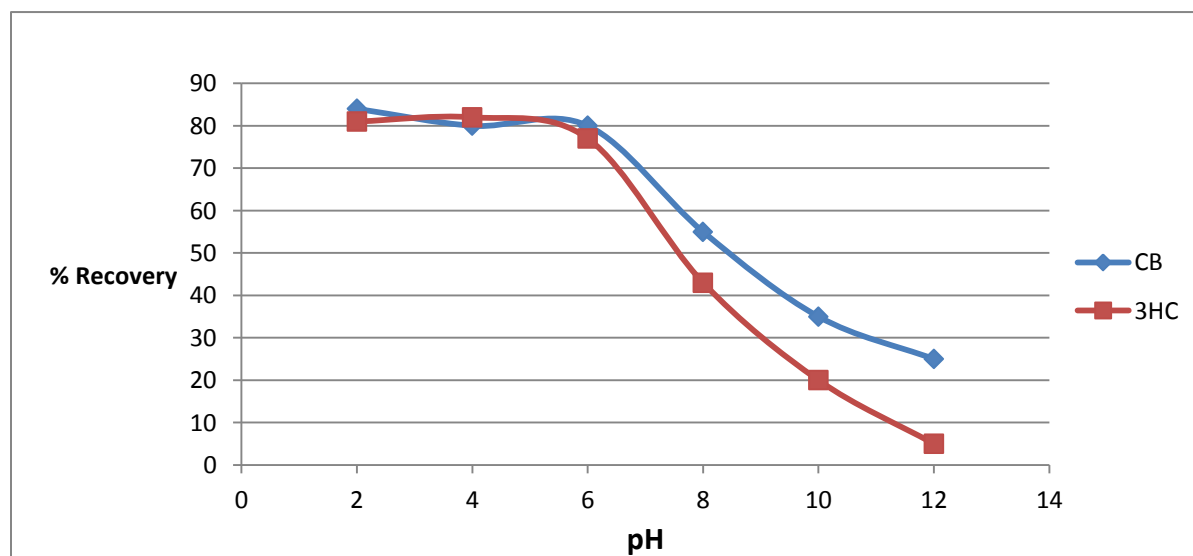


Figure 3.9: Alkaline hydrolysis induced curve for CB and 3-HC

At lower pH levels i.e. 2-6, 3-HC recoveries were not significantly different ($p < 0.05$) from the once observed in CB and were between 77-81%. At neutral point, 3-HC yielded recoveries of 72%. A sharp decline was observed in the recoveries realized with 3-HC above pH 7.5. Osman and Eldib (1991) in a study of of sevin and baygon which are carbamate pesticides observed that the compounds were stable to hydrolysis within the acidic pH range which agrees with the results from this study. They found measurable hydrolysis was as from pH 7.0. The decline observed from pH 7 in both CB and 3-HC also agrees to Peggy and Wayne (2005) who were able to also show that many pesticides, particularly commonly used organophosphate and carbamate insecticides, undergo a chemical reaction in the presence of alkaline water that reduces their effectiveness in a reaction is that called alkaline hydrolysis. The pesticide is hydrolyzed and rendered ineffective when it is mixed with water with a pH greater than 7. The more alkaline the water, the more rapidly the pesticide breaks down. Ferrell and Aagard (2003) too observed that the hydrolysis rate can be rapid in a pH range of 8 to 9 and that for every pH point increase; the rate of hydrolysis will increase by a factor of about 10. At pH 12, 3-HC did give a recovery of 5% which is far much lower compared to CB which was 22 %. The difference between the CB and 3-HC can be attributed to the active functional groups in the structure of the two compounds. 3-HC has an OH functional group thus experiences a much more rapid hydrolysis. Hydrolysis reactions occur due to both H^+ and OH^- ion, however base catalysis i.e. due to OH^- ion which is a much more reactive nucleophile than H_2O , and thus an increase in pH which means an increase in OH^- increases the rate of hydrolysis (Osman & Eldib, 1991)

3.7 Pesticide determination on field samples

The identification and separation capabilities of GC can be increased when combined with the confirmation capabilities of the MS. MS is unique in that it not only serves as a qualitative detector, but is equally powerful as a quantitative detector providing detailed structural information (Juan *et al.*, 2007). The eluted compounds were identified via the GC-MS NIST library. The pesticide monitoring study did not confirm the presence of carbofuran, any of its metabolites or degradation products in all of the analysed samples. However, two organophosphate pesticides – diazinon and fenitrothion were detected in soil and paddy water samples. The two organophosphate pesticides

were detected in three of the ten soil samples (from Wamumu and Karaba A & B). Figure 3.10 and 3.11 show selected TICs of soil and water samples. Fenitrothion eluted at 16.83 minutes (Figure 3.10) while diazinon eluted at 14.99 minutes (Figure 3.11).

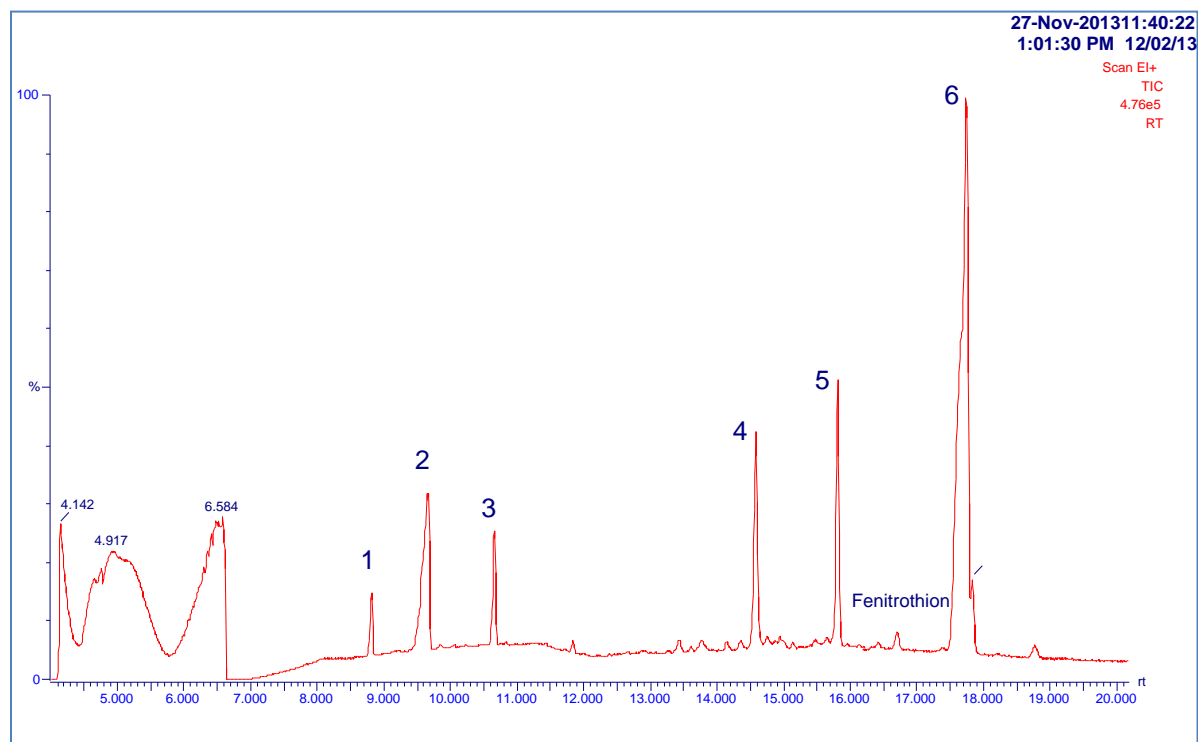


Figure 3.10: A TIC for a soil sample showing elution of fenitrothion at 16.83 minutes (Other peaks- 1: Isopropyl ester at 8.79 minutes, peak 2: 5-Ethyl-3-Methyl-1-en-4-ol, at 9.67minutes; peak 3: Permetrinic acid at 10.67 minutes, peak 4: Benzene acetonitrile at 14.75 minutes, peak 5: Palmitic acid at 15.86 minutes and peak 6: Benzene dicarboxylic acid at 17.77 minutes)

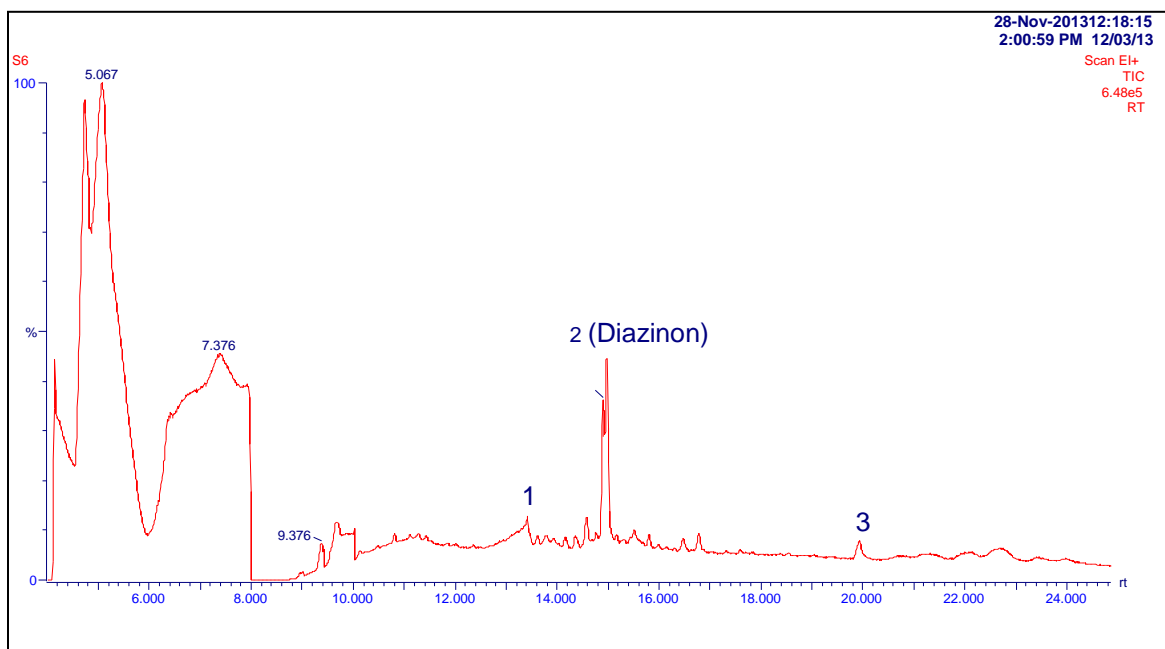


Figure 3.11: A water sample TIC showing elution of diazinon at 14.99 minutes
 (Other peaks- peak 1: 2-Nonane at 13.6 minutes and 3: Tetradecanoic acid at 20.00 minutes)

The presence of the other noted peaks can be attributed to various components in the sample matrix. Isopropyl ester and ethyl-3-methylhept-1-en-4-ol are industrial solvents used in the manufacture of chemicals. Permetrinic acid is a compound used in manufacture of cypermethrin –a pyrethroid pesticide. Palmitic acid and tetradecanoic acid are common fatty acids found in plants while benzenedicarboxylic acid is a plasticizer, and a contaminant sometimes observed in GC-MS analyses.

Figures 3.12 and 3.13 illustrate the mass spectra of fenitrothion and diazinon respectively.

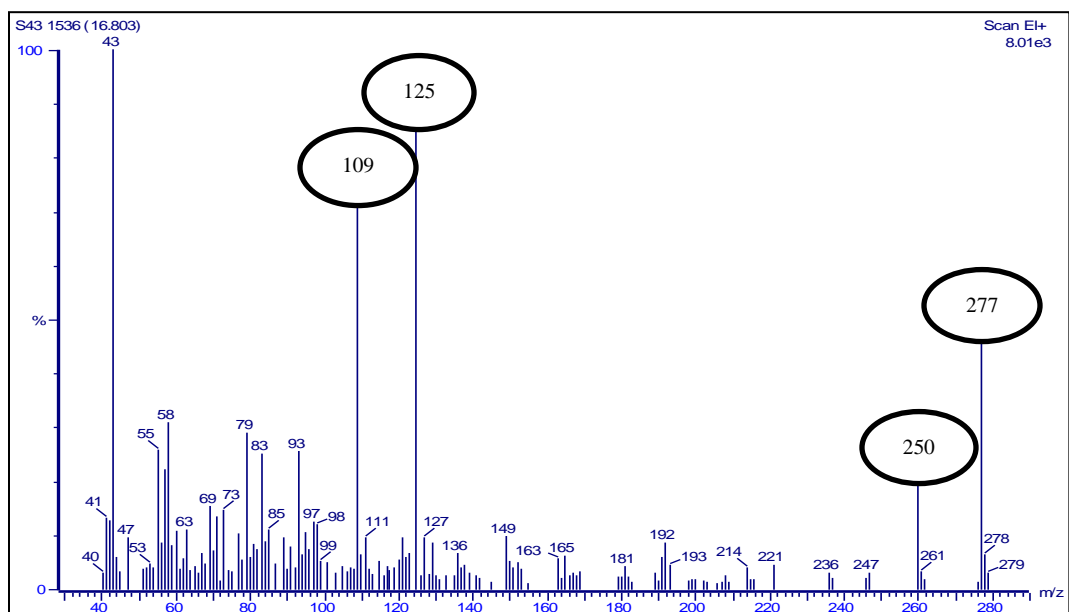


Figure 3.12: Mass spectrum of fenitrothion

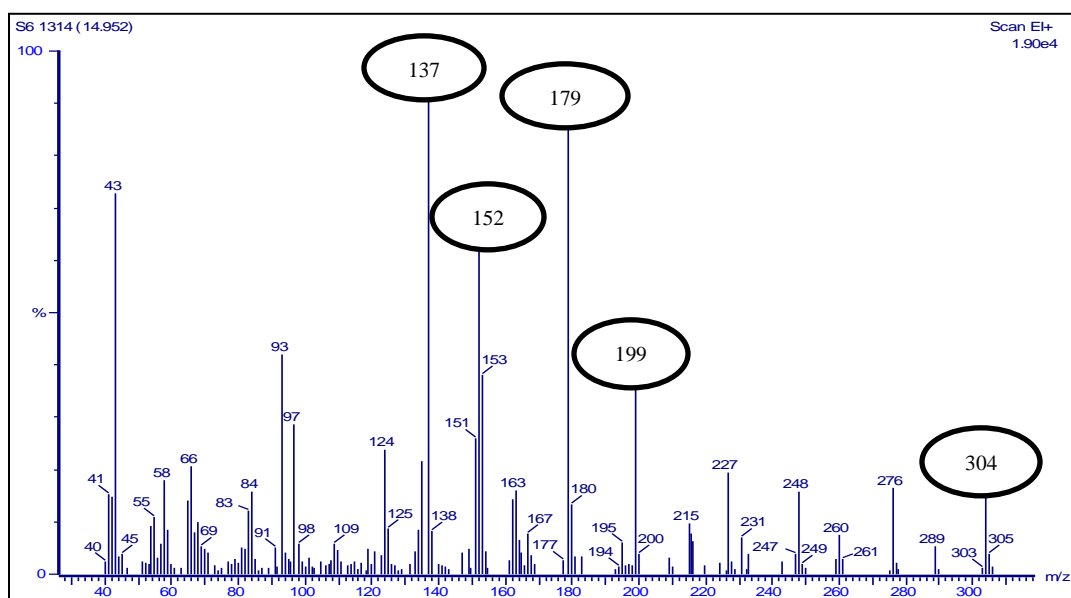


Figure 3.13: Mass spectrum of diazinon

Selected ion monitoring mode (SIM) was used in the identification and quantification of the two pesticides (Table 3.10).

Table 3.10: Monitor and quantification ions

.Pesticide	Monitor ions	Quantification ion
Carbofuran	149,164,221	164
3-hydroxycarbofuran	137,147,237	147
Diazinon	137, 179, 152, 199, 304	137
Fenitrothion	109, 260, 277	125

Detection limits for diazinon and fenitrothion were 0.11 and 0.17 $\mu\text{g/ml}$ respectively. The concentration of diazinon in the soil sampled from Wamumu A was 0.24 mg/kg. Fenitrothion concentrations in soils from Karaba A and B were 0.06 mg/kg and 0.11mg/kg respectively as shown in Table 3.11. The only water sample from the paddies in which a pesticide was detected was sampled from Wamumu section A. Diazinon residues of 0.19 mg/L were quantified from this sampling site. No pesticide residue was detected in either the rice plants or the water from the channels feeding the irrigation paddies

Table 3.11: Pesticide residue levels in paddy water and soil samples

Sampling site		Diazinon		Fenitrothion	
		Water	Soil	Water	Soil
Mwea	A	<BDL	<BDL	<BDL	<BDL
	B	<BDL	<BDL	<BDL	<BDL
Thiba A	A	<BDL	<BDL	<BDL	<BDL
	B	<BDL	<BDL	<BDL	<BDL
Thuba B	A	<BDL	<BDL	<BDL	<BDL
	B	<BDL	<BDL	<BDL	<BDL
Wamumu	A	0.19 mg/L	0.24 mg/kg	<BDL	<BDL
	B	<BDL	<BDL	<BDL	<BDL
Karaba	A	<BDL	<BDL	<BDL	0.06 mg/kg
	B	<BDL	<BDL	<BDL	0.11mg/kg

It was noted that these two pesticides were detected in the low lying paddies- which does not discount the possibility that a portion of the pesticides in the upper paddies migrated to and were eventually deposited there. Musa *et al.*, (2011), in a study carried out in lower Nyando, Kenya, (which is a rice growing area) was able to detect diazinon and malathion in sediment samples at levels of 0.56-1.08 µg/kg. The water samples did not contain any detectable levels of organophosphorus pesticides.

Akan *et al.*, (2013) in the Alau Dam and Gongulong Agricultural areas of Nigeria detected diazinon and fenitrothion in onions, spinach and soil. The diazinon residue levels in onions and spinach leaf was at 20 µg/g and 18 µg/g respectively while fenitrothion concentration was at 26 µg/g and 28 µg/g respectively. The residue levels in the soil at the region were at 100 µg/g and 95 µg/g for diazinon and fenitrothion respectively at 11-20 cm sampling depth. These concentrations far exceed the ones quantified in this study. The results observed in Akan *et al.*, (2013) study, are a reflection of the systemic nature of organophosphate pesticides. Elsewhere, carbofuran and diazinon pesticide residues in levels of up to 198.7 µg/L and 0.9 µg/L

respectively in water have been reported in paddy fields in Savar Upazila, Bangladesh (Chowdhry *et al.*, 2012).

Carbofuran has a half-life of 40 days at pH 7 and a half-life of 3 days at pH 9. This is an indication of its susceptibility to alkaline hydrolysis. Diazinon on the other hand has a half-life of 70 days at pH 7 which drops to 29 days at pH 9 (Ferrell & Aagard, 2003). This reflects that organophosphates can be more persistent and better withstand environmental breakdown – a probable reason why only organophosphates were detected.

Nonetheless, the organophosphate pesticide residues detected in the paddy water and soil samples taken for this study were above the accepted maximum levels of total and individual pesticide contamination, at 0.5 and 0.1 µg/L, respectively, in water as recommended by the European Economic Commission (Directive 98/83/EC) (EEC, 2010).

CHAPTER FOUR

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

A simple, clear validated and optimized method for the determination of organophosphate and carbamate pesticides was developed. However, no carbamate pesticide was detected from the sample analyzed only two organophosphate pesticides; diazinon and fenitrothion were detected and quantified.

The use of fast GC column Db-X1b which has a narrower internal bore and thinner adsorption film proved to be worthwhile since clear separation of compounds of interest was observed coupled with the lower LOD and LOQ values it yielded. The GC-MS system was stable enough since both repeatable and reproducible results were obtained during the method validation processes. Employment of the SPE method of extraction was beneficial since higher recoveries were obtained and minimal quantities of organic solvents were used in the extraction procedures.

pH was observed to be a vital physico-chemical parameter in determining the availability of carbamate pesticides in the environment. There was significant loss of the active ingredient of carbamate pesticide when introduced to environment with pH values lower than 7.

Based on the residue results from this study, there is a high probability that other water sources will be contaminated as the irrigation waters exit the rice scheme. Care should be taken in the application of these pesticides to minimize their impact on the environment. The concentration levels of the two pesticides i.e. diazinon and fenitrothion in the environment were above the set MRLs by EEC.

4.2 Recommendations

4.2.1 Recommendations from this study

- i. Since appreciable levels of the pesticides were detected, farmers should be tested to ascertain for pesticide levels in their bodies.

- ii. Drinking water sources in the area be tested for contamination from pesticide residues.

4.2.2 Recommendations for further studies

- i. Follow up studies should be conducted during the rainy season to monitor any differences in the residue levels and also determine presence of other pesticides
- ii. A degradation kinetics study on pesticides detected when exposed to change in pH and other physico-chemical parameters.
- iii. Continuous monitoring studies to ascertain the pesticides used in the rice farms.
- iv. Further studies could be conducted using higher precision instruments with much lower detection limits, such as HPLC-MS to ascertain the absence of the pesticides such as carbamates which were not detected in this study.

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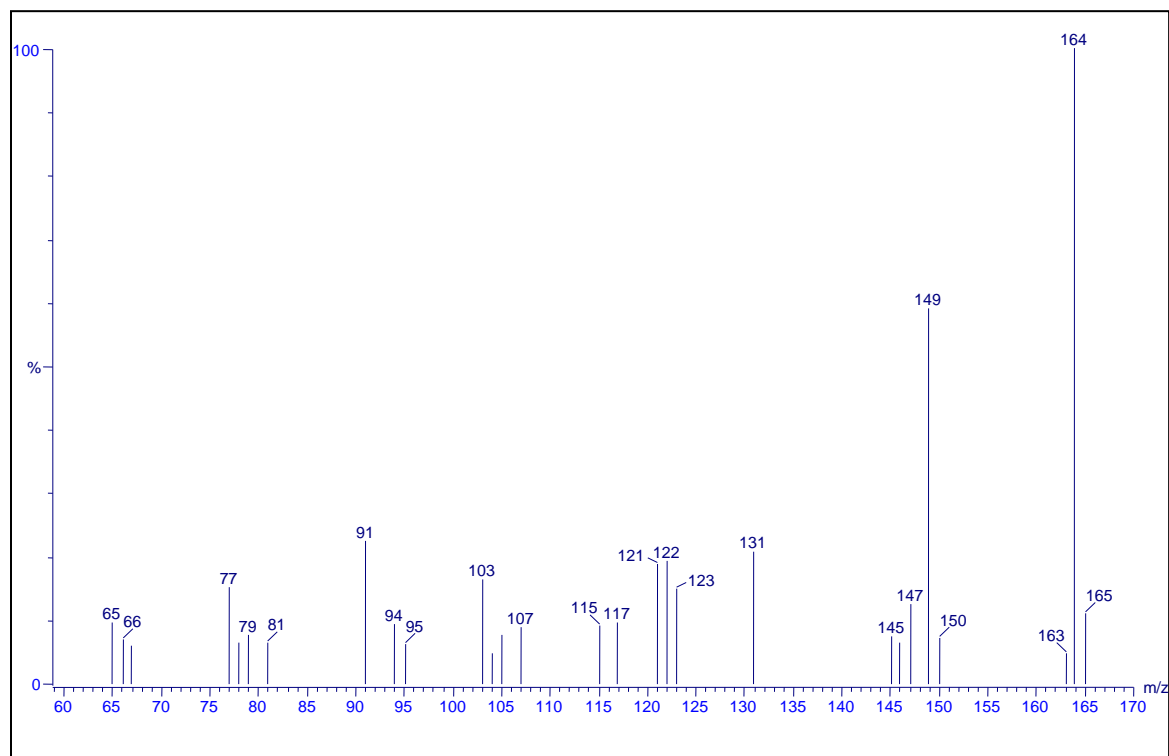
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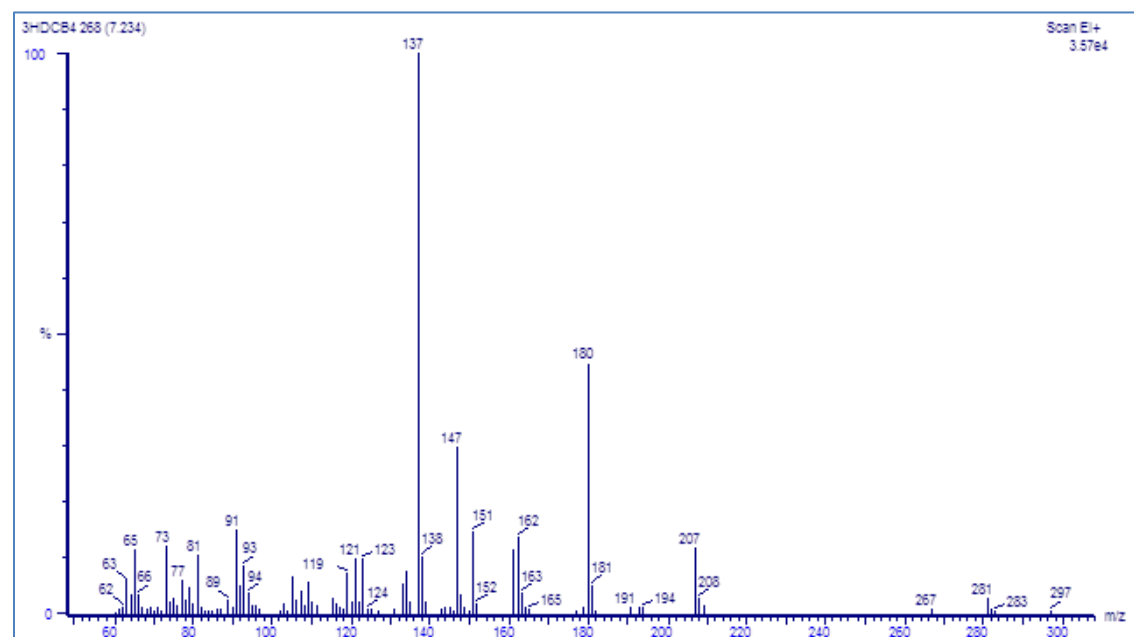
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5.0 APPENDICES

Appendix 1 : Mass spectrum of carbofuran showing loss of parent ion (loss of m/z 221)



Appendix 2: Mass spectrum of 3-HC indicating loss of parent ion



Appendix 3: Mass spectrum of 3-hydroxycarbofuran

