UNIVERSITY OF NAIROBI

DEPARTMENT OF CHEMISTRY

DETERMINATION OF PRE-HARVEST INTERVAL FOR CARBENDAZIM USED FOR CONTROL OF FUNGAL DISEASES IN FRENCH BEANS

BY

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DECLARATION

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

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ABSTRACT

French beans export contribution to the economy of Kenya has seen it become the second foreign exchange earner after tea; providing employment, increased food security and sustainable development in the country. In the process of crop production, farmers use pesticides to control pest and diseases to improve the yield and quality of the produce. Pre-Harvest Interval (PHI) is observed to reduce the risk of human exposure to pesticide carry over through food. In this study the PHI of carbendazim in French beans was evaluated in a supervised field trial conducted in Kambaa and Naivasha regions in Kenya. The data collected through a questionnaire showed that exporters of French beans deal with more than one variety and were aware of fungal diseases affecting French beans. To control the pest, exporters uses pesticide such as carbendazim. The field trial was conducted in the months of September to November, 2015 and March to May, 2016. Pesticide under the study was applied at the rate of 625g of carbendazim per hectare before harvest and samples collected at 0, 3, 7, 14 and 16 days after application. Laboratory samples were analysed for presence of carbendazim residues using Liquid Chromatography Quadruple (Agilent 6430 LCMSMS) with standard Electron Spray Ionization (ESI).The study revealed that in the short and long rain seasons, the breakdown of carbendazim in French beans followed a similar pattern of residue degradation irrespective of the formulations, season or location. Degradation in the first three days after the pesticide application was sharp ranging from 36.4% and 60.6%. The results showed that the pesticide residue degraded to below the European Union (EU) set maximum residue limit (MRL) of 200µg/kg at 7 days after application. Degradation of carbendazim in French beans follows Langmuir-Hinshelwood kinetic equation and a dissipation half-life of carbendazim in French beans was found to be 1.7 days. A PHI of 5 days was found to be sufficient for carbendazim if applied in the recommended rate of 625g per hectare with all Good Agricultural Practice (GAP) observed. A National Tolerance Level of 74µg/kg was proposed to be used by the relevant government authorities as a buffer level for compliance monitoring.

DEDICATION

This thesis is dedicated to the Almighty God, my creator and provider. To Him alone be all the praise and glory for giving me the opportunity, knowledge and wisdom in my studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

APVMA	Australian Pesticides and Veterinary Medicines Authority.
CAC	Codex Alimentarius Commission.
EC	European Commission.
EFSA	European Food Safety Authority.
EU	European Union.
FAO	Food and Agriculture Organization of the United Nations.
GAP	Good Agricultural Practice.
HCD	Horticultural Crop Directorate.
HCDA	Horticultural Crop Development Authority.
HPLC	High Pressure Liquid Chromatography.
IPC	International Programme on Chemical Safety.
IPM	Integrated Pest Management.
KEPHIS	Kenya Plant Health Inspectorate Service.
KNBS	Kenya National Bureau of Statistics.
LCMSMS	Liquid Chromatography Triple quadruple Mass spectrometer.
LD	Lethal Dose.
MoALF	Ministry of Agriculture Livestock and Fisheries.
MRLs	Maximum Residue Limits.
PAN	Pesticide Action Network Europe.
PCPB	Pest Control Products Board.
PHI	Pre-Harvest Interval.
PPP	Plant Protection Product.

PSA	Primary Secondary Amine.
RASFF	Rapid Alert System for Food and Feed.
SC	Suspension Concentrate.
SI	System International (International System of Units)
SNV	Netherland Development Organization.
SOP	Standard Operation Procedure.
USAID-KAVES	United States Agency for International Development- Kenya Agricultural Value Chain Enterprises.
WHO	World Health Organization of the United Nations.
WP	Wettable powder.

UNITS OF MEASUREMENT

g	Grams.
Ha	Hectare.
H_0	Null hypothesis.
Kg	Kilogram.
m	Meter.
mg	Milligrams.
ng	Nano gram.
ppm	Parts per million.
rpm	Revolution per minute.
µg	Microgram.
µl	Microliter.
v	Volume.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background.

Agriculture sector plays a fundamental role in the economy of Kenya. Under the economic pillar of vision 2030 (Ministry of Planning and National Development, 2012), Agriculture is identified as one of the key sectors to deliver the 10% growth per year (Ministry of Planning and National Development, 2012). The sector supports over 70% of the Kenyan population living in the rural areas who are engaged in farming as their main source of livelihood. Therefore the agriculture sector continues to play an overwhelmingly important role in sustainable rural development in Kenya. In 2014, Agriculture sector contributed 27.3% of the GDP and recorded a positive growth of 3.5% (KNBS, 2015).

French bean is a vegetable crop under the horticultural sub-sector of Agriculture sector. The sub-sector has grown significantly since 2000 to become a major foreign exchange earner to the country. Its contribution to the economy of Kenya has seen it become the second foreign exchange earner after tea; providing employment, increased food security and sustainable development in the country. In 2012, the total value of horticultural exports from Kenya was Ksh. 87 billion (Euros 852 million); most of the produce going into the Europe market (HCDA, 2014). French bean is a major vegetable in the sub-sector grown mainly for export, with EU being the biggest market. In Kenya, small scale farmers in the rural areas are the primary producers of fruits and vegetables for both local and export market. French bean is a relatively high value vegetable with a high return per hectare, short growth cycle and minimum capital investment. This makes French beans become a preferred commercial crop of choice for many small scale farmers, estimated at 50,000 famers (Netherlands Development Organization (SNV), 2012).

French beans are leguminous plants in the species *Phaseolus vulgaris L* which are important components in human diets throughout the world (Kenneth, 2012). Apart from human consumption they are also used as animal feeds, for improving soil conditions and nitrogen fixation in the soil. The young immature pods of the French beans are harvested and consumed as vegetables. Their nutritional value ranges from protein, iron and essential nutrients such as

ascorbic acid, vitamin B, and calcium (John *et al.,* 2011). The total production of French bean in 2014 was 112,409 tons valued at Ksh 5.04 billion (HCD, 2014).

French beans, also known as green beans, string beans or snap beans, originated from Peru, and spread to South and Central America by migrating Indian tribes. It grows very well in warm climatic conditions with temperatures between 21^0C-27^0C (John *et.al.* 2007). Developed countries are the major consumers of French beans and therefore form the main market (Julius, 2005a). French beans has increasingly become an important commercial crop to small scale farming communities in the rural areas and its production supports more than 1 million people in the entire value chain (HCDA, 2011). It has become the second largest vegetable export in Kenya, contributing over 60% of exported vegetable in 2011 (HCDA, 2011). United Kingdom, Germany, France and Netherlands are the main EU market for French beans with small quantities being exported to Middle East among other countries. Tanzania, Uganda and Rwanda are other East Africa countries with the potential to produce French beans (Horticultural Insights, 2006).

The geographical position of Kenya in the tropical zone gives her favorable climatic conditions for the growth of French beans throughout the year with high quality attributes such as softness, non-stringy fibrous texture and compatibility with other vegetables. The leading counties producing French beans in Kenya are Kirinyaga, Murang'a, Meru and Machakos accounting for 47%, 25%, 14% and 9% respectively of the total production (HCD, 2014). In these areas, French beans are produced predominately by small scale farmers especially women and youth; farming forms a major source of their income (John *et al*, 2011). According to Horticultural Crop Directorate report, the area for production decreased from 4,707 Ha in 2013 to 4,572 Ha in 2014, while the yields and value increased by 9% from 112,409 tons to 122,666 tons and 15% from 4,382 million to 5,038 million, respectively (HCD, 2014). Europe is a major destination of Kenyan exported French beans, a success based on the country's climatic and geographic competitive advantage and adaptation of certification schemes (USAID-KAVES, 2015).

The favorable climate for the growth of French beans coupled with global climate change due to human activities has contributed to numerous types of pests and diseases in Kenya. This has led to an increase in the use of pesticides to control pest and diseases to improve the yield and quality of the produce. To meet the European Union food safety standards with the use of pesticides has become challenging to the small scale farmers. This is because of the quality

standards that are to be met, thus increasing the fixed cost and transaction's cost of producing French beans (Julius, 2005b).

The concern of pesticide residues in or on food to the health of consumers and environmental contamination has led to establishment of food safety standards and protocols such as Maximum Residue Limits and pesticide usage which growers are required to meet (European Commission, 2010). The main objective of these regulations is to minimize the levels of exposure of consumers to pesticides, reduce the risk and as a result assuring food safety without significantly affecting trade. EU has one of the best developed pesticide residue regulations and system controls for food safety. Kenya's horticultural produce exports to EU are subjected to these regulations to ensure compliance with food safety requirements (European Commission, 2010).

The most recent impact of the EU regulation in Kenyan export is the December 2012 European Commission decision to subject French beans imported from Kenya to pesticide residue testing for compliance before entry to EU market. The decision was based on the EU regulation (EC) 882/2004 on official controls performed to ensure the verification of compliance with feed and food law. From January 2013 to 31st June 2015, in accordance with Annex 1 of the regulation, (EC) 669/2009 implementing the decision, French beans consignments imported from Kenya would be subjected to a 10% sampling for testing at the official designated port of entry. Ideally the consignment would be detained at the port of entry for 1-3 days awaiting the release of the laboratory analysis results. The consignment would be allowed entry if it complied with the EU food law or denied entry and destroyed where it did not comply. This led to loss on produce shelf life and decrease in competitiveness of the Kenyan French beans in the international market and also the associated costs were passed on to the farmer, which reduced the returns to the farmers.

The impact of listing Kenyan French beans with pods in EU regulation (EC) 669/2009 were; loss of shelf life, decreasing the competitiveness of Kenyan beans with pods, loss of income due to extra charges brought about by change of routes to ensure entry of consignments through EU designated ports as per the regulation (EC) 882/2004 for inspection.

Good Agricultural Practices (GAP) is a standard procedure which when practiced by farmers, greatly improve the levels of compliance of Kenya's Agricultural produce with market requirements. Integrated Pest Management (IPM) and Pre-harvest interval (PHI) are critical components of GAP. It is also important to respect the PHI so that the MRLs for a given crop

is not exceeded. Residues found in excess of the MRLs on or in food would constitute a violation of the WTO Sanitary and Phytosanitary Standards (SPS) agreement and could pose a risk to consumers' health and access to market.

The pre-harvest interval (PHI) is a function of a pesticide's use pattern and of the amount of pesticide residues allowed on the crop at harvest. Residue levels on a crop are affected by the crop's growth (Pesticide Management Regulation Agency of Canada, 2007), by environmental conditions (such as rain or UV radiation) and by the microorganisms on the plants and in the soil. The PHI must therefore be long enough to allow for the pesticide residues in the harvested crop to degrade to a level that is legally acceptable. It is important to observe the PHI to ensure that the established MRL tolerance for a given crop is not exceeded. Residues found in excess of the MRL on or in food would constitute to violation of the Food and Drug Regulations and could also pose a risk to consumers' health (Pesticide Management Regulation Agency of Canada, 2007). Pesticides should be used only for the crops and pests listed on the product's label and make sure to follow the application rates, number of applications and PHI stated on the label. It is a legal requirement in Kenya for all registered plant protection products to have an established PHI which appears on the label of the product (CAP 346, 2012). PHI is established through supervised field trials under the climatic conditions and application of the pesticide in the recommended dose as per the registration dossier. According to PCPB 9th edition of registered plant protection product in Kenya; Bendazim 500 SC, Chariot 500 SC and Rodazim SC are the only single molecule carbendazim formulation registered in Kenya. However, this formulations have different pre-harvest interval as shown in table 1.1.

Table 1.1: Carbendazim formulations registered in Kenya for foliar use in French beans.

Serial No.	Trade Name and Type of Formulation	Registration number	Active ingredient (Common name(s)	Manufacturer	PHI (Days)
1	Bendazim 500 SC	PCPB (CR) 0378 p(i)	Carbendazim 500g/l	Rotam Agrochemicals, Hong Kong	No PHI
2	Chariot 500 SC	PCPB (CR) 1084	Carbendazim 500g/l	Ningbo Yihwei chemical Co.Ltd China	PHI 5
3	Megaprode Lock 52.5 WP	PCPB (CR) 1133	Carbendazim 17.5g/kg + Iprodione 52.5g/kg	Jiangsu Kuaida Agrochemical Ltd	PHI 14
4	Pearl 500 SC	PCPB (CR) 0545	Carbendazim 500g/l	Sulphur Mills Ltd, India	No PHI
5	Rimeta Gold 300 SC	PCPB (CR) 1228	Pyrimethanil 5% w/w + Carbendazim 25% w/w	Yantai Kenda Chemicals Co. Ltd China	PHI 7
6	Rondazim SC	PCPB (CR) 0378	Carbendazim 500g/l	Rotam Ltd, Hong Kong	PHI 7
7	SAAF WP	PCPB (CR) 0717	Carbendazim 12.25% w/w + Mancozeb 74% w/w +	UPL Ltd, India	No PHI
8	Exempocurve 250 SC	PCPB (CR) 126	Expoxiconazole 125g/l + Carbendazim 125g/l	Green life crop protection Africa Ltd	No PHI
9	Sherrif 75 WP	PCPB (CR) 0717 p(i)	Carbendazim 12.25%w/w + Maconzeb 74%w/w	UPL Ltd	No PHI

Source: PCPB 9th edition of registered plant protection product in Kenya.

Potential French beans diseases in Kenya include Damping-off, Bacterial Blights, Rust, Angular leaf spot, Anthracnose, Botrytis, and virus. The type of disease or virus determines the plant protection product to be used by the farmer. According to pest control ACT CAP 346 (Revision, 2012) of the laws of Kenya, a farmer can only use plant protection products registered in Kenya by the Pest Control Products Board (PCPB). Carbendazim is a chemical known to control Angular leaf spot, Anthracnose and Botrytis in French beans. The compound has been formulated as a Suspension Concentrate (SC), Wettable Powder (WP) or Emulsifiable Concentrate (EC) formulations. The formulations in Table 1.1 are registered in Kenya and listed by PCPB 9th edition of registered pesticides for use in control of the fungal diseases in French beans.

1.2 Statement of the problem.

The use of pesticides during the active growth of the crops enables farmers to produce some crops in areas that otherwise would not be suitable, control the growth of weeds, enhance crop yield, preserve produce quality and extend shelf life. Geographically Kenya is located in the tropical zone which has a favorable climate for numerous pests and diseases. Therefore the use of pesticides play a major role in ensuring the availability of food to the rapidly increasing population in the world.

However, pesticides and their metabolites or breakdown products are chemicals which can present health risks to the people who apply them, consumers, and non-target organisms and to the environment when not used properly (Sitaramaraju1, 2014). Pesticide enters in the food chain as pesticide residue after their use in control of pest and diseases. Therefore, food security; food safety and sustainability of the environment for crop production have prompted countries to establish regulations and standards for the safe production of crops which can be safely consumed. European Union is a major trading partner of Kenya in fresh vegetables with well-established regulation to protect consumer and environmental health. To access the European Union market, French beans from Kenya must not contain pesticide residues at or above the Maximum Residue Limits (MRLs) set in EU regulation (EC) 396/2004. According to Pesticide Action Network Europe (PAN) fact sheet, carbendazim is a dangerous toxin capable of causing malformation of foetus at very low doses and being harmful to aquatic organisms (Pesticide Action Network Europe (PAN), 2014). In 2014 the EU standing committee approved its ban (Pesticide Action Network Europe (PAN), 2014) under the rules of European Union in regulation (EU) 1107/2009.

Formulations containing Carbendazim are registered in Kenya for seed dressing and foliar use. Therefore, where this pesticide has been used in the crops protection, pre-harvest interval (PHI) must be observed in order to avoid maximum residue limits exceedances. This will ultimately ensure EU market access, minimise border rejection and improve economic development in Kenya. French beans diseases such as Rust, Angular leaf spot; Anthracnose and Botrytis are common in Kenya. These diseases are majorly controlled by the use of carbendazim based pesticides. Kenya does not have a harmonized PHI for registered pesticide formulations containing carbendazim.

1.3 Overall objective.

The general objective of this study was to determine the pre-harvest interval (PHI) of two carbendazim formulations registered in Kenya for control of fungal diseases in French beans to ensure compliance with EU maximum residue limits by conducting supervised field trials.

1.3.1 Specific objectives

The specific objectives were to:

1) Determine the dissipation rate of two Carbendazim formulations (Chariot 500 SC and Rodazim SC) in French beans.
2) Determine pre-harvest Interval (PHI) for the two Carbendazim formulation in French beans.
3) Propose a National MRL tolerance for carbendazim in French beans.

1.3.2 Hypothesis (Ho).

Harmonized carbendazim PHI does not reduce the incidences of non-compliance of French beans exports to EU due to MRLs exceedance.

1.4 Justification and significance of study.

Given the high value and growth cycle of 45-60 days, French beans has the potential to have three crops in a year (USAID-KAVES, 2015) which assures the farmer a consistent cash flow. According to USAID-KAVES French beans value chain analysis, 'The supply chain is estimated to engage 50,000 small-scale farmers and employ between 45,000 and 60,000 people depending on the season.' In the process of production, pesticides (herbicides, insecticides, fungicides, Nematocides, fertilizers and soil amendments) are used to prevent pest damage and increase yield. Ideally, pesticides are applied to harm the target organism. Unfortunately, this

is not the case giving a possibility of pesticide entering into the food chain (Harsimran et al., 2014).

Carbendazim is one of the pesticides used to control Angular leaf spot; Anthracnose and Botrytis in French beans. Carbendazim is a systemic benzimidazole fungicide that is used to control fungal diseases on pulses, fruits, macadamia nuts, cucurbits, pastures, roses, timber and turf; it is also used in post-harvest storage of fruits (Australian Pesticide and Veterinary Medicine Authority, 2007). Carbendazim is both a metabolite and breakdown product of benomyl and thiophanate-methyl in plants and the environment. Carbendazim is registered in Kenya for use in control of fungal diseases in horticultural produce (beans with pod, peas with pods and roses). According to European Commission, carbendazim is believed to affect hormone function (Pesticides News, 2002) and is capable of causing malfunctions in the foetus at very low doses (Pesticide Action Network Europe (PAN), 2014).Trend analysis of analytical data for laboratory results and notification from EU Rapid Alert System for Food and Feed (RASFF) in 2014 and 2015 from Kenya Plant Health Inspectorate Service (KEPHIS) show that carbendazim residues detected in beans and peas with pods has been on the increase. Most of these detected cases are below the Maximum Residue Level of the EU. There are nine (9) carbendazim plant protection products registered in Kenya for use in control of fungal diseases in beans with pods. However, the Pre-Harvest Interval (PHI) varies between zero (0) to fourteen (14) days in the different brands listed in the Table 1.1 (PCPB, 2015).

Pre-harvest intervals is the time between the last application of a plant protection product and harvest or the earliest possible use of the treated product. PHI is part of Good Agricultural Practice which must be observed to reduce the risk of human exposure to pesticide. The huge variation in the PHI in the various brands may be contributing to the increase in its detection in the produce. Therefore establishment of a harmonized PHI is paramount since the continuous changes in EU MRLs may cause carbendazim to be included in the list of banned or controlled use pesticide substances in the future.

Subsequently there is no study in Kenya that has been done to harmonize PHI for similar molecules in different formulations. The current study therefore, determined the dissipation rate of carbendazim in French beans thereby determining a suitable PHI for carbendazim formulation registered in Kenya. The information obtained in the study will be shared with the relevant government bodies for consideration during designing and implementation of the national risk based pesticide residue surveillance at farm level and exit point.

The data obtained in the study will be used to determine a suitable PHI for carbendazim in French beans from Kenya exported to EU market. The data will be shared with KEPHIS, Ministry of Agriculture Livestock and Fisheries (MoALF) and other relevant authorities for policy formulation and designing of risk based surveillance at farm level and point of exit as a sustainable measure taken by Kenya to control pesticide residue non-compliances and therefore increase compliance to market requirements of Kenya produce.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of French beans.

French beans (*Phaseolus vulgaris L*) or Snap beans (Figure 2.1) originated in tropical Southern Mexico, Guatemala, Honduras and Costa Rica (Craig, 2003). French beans are in the class of bush beans known to stand erect without support and consumed as immature pods. They have high yield and require minimum amount of work (Craig, 2003). Pest and diseases present major constraints in production of French beans by small scale farmers in Kenya. Yield losses due to pest or rejections due to pesticide residues detected in produce above maximum residue limits are some of the constraints in the French bean exportation.

Figure 2.1: French beans crop (Source: SACCO)

2.2 French beans grown in Kenya.

The French bean is a major vegetable crop grown in Kenya for export (Nderitu *et al.,* 2007) mainly targeting European Union market. In Kenya, the crop ranks first among the vegetables produced for export market accounting for a significant proportion of total horticultural exports (HCDA, 2009). In 2014, production of French beans by small scale farmers was 112,409 tons

valued at Ksh 5.04 billion, which was an increase by 15% growth compared to 2013 (HCD, 2014). The geographical position of Kenya in the tropical zone gives her favorable climatic conditions for the growth of French beans throughout the year. The crop is grown largely under rain fed cultivation and irrigation as supplement to ensure continuous productions at low rainy seasons. Locally, the vegetable is not commonly consumed; however there has been a rapid increase in the number of small scale farmers adopting its farming as a cash crop for export. French bean has a short crop cycle of 45- 60 days (USAID-KAVES, 2015) with minimum capital investment. French bean farming provides a source of income among the youth and women in the rural areas and therefore addresses the issue of food insecurity, unemployment and poverty alleviation.

2.3 French beans farming in Kenya.

In Kenya, French beans are referred to as *mishiris* are a major vegetable that dominate Kenya's export market with a low local consumption rate. The French beans grows at optimum temperatures of 20^0C -25^0C and at an altitude ranging from 1200-2100 meters above sea level in a well distributed medium to high annual rainfall of about 900-1,200mm per year. To ensure continuous production throughout the year, supplementary irrigation of up to 50mm of water is applied per week where the rainfall is not adequate.

Depending on the variety, French beans can survive in temperatures ranging from 14^0C -32^0C. Extreme temperatures can result into poor flower development and pod formation. An optimum soil pH of 6.5-7.5 is recommended (USAID-KAVES, 2015); however, French beans can tolerate a low pH of up to 4.5 (USAID-KAVES, 2015);.A pH below 4.5 may result into an impaired growth due to poor development of the rhizobium bacteria which is responsible for nitrogen fixation in beans (Carol, 2016). The French beans are grown mainly in warm areas of Kenya, which include: Kirinyaga, Meru, Machakos, Narok, Kiambu, Embu, Murang'a, Taita Taveta, Nyeri, Bomet, Makueni and Kajiado counties. Kirinyaga County is the leading producer of French beans in the country accounting for 47% of the total produce for export (HCDA, 2014), (Table 2.1). High season for the export of French is between September and March during the winter weather in EU, while low season occurs between June and September during summer in the EU.

Table 2.1: Counties producing French beans in Kenya, under small scale farming.

	2012			2013			2014		
County	Area (ha)	Quantity (ton)	Value Kshs (Million)	Area (ha)	Quantity (ton)	Value Kshs (Million)	Area (ha)	Quantity (ton)	Value Kshs (Million)
Kirinyaga	1,813	51,148	2,455.58	1,481	45,626	2,053.78	1,536	47,440	2,372.81
Murang'a	861	3,848	1,185.28	885	36,810	1,268.21	847	34,690	1,268.10
Meru	326	16,615	616.63	367	13,328	530.32	407	17,030	681.33
Machakos	326	1,760	75.22	522	2,415	106.01	398	11,139	433.15
Narok	105	1,575	94.5	120	900	54	120	900	54
Kiambu	221	4,149	55.95	226	3,832	45.83	191	3,749	47
Taita Taveta	48	1,191	42.23	134	3,514	147.59	58	1,245	43.74
Embu	58	746	25.85	43	639	34.33	35	490	26.03
Nyeri	139	428	623.85	148	431	9.38	143	525	16.36
Bomet	-	-	-	-	-	-	54	240	13.68
Makueni	74	379	16.52	62	376	16.43	97	421	13.68
Kajiado	88	478	17.04	95	580	25.38	81	421	13.09
Others	894	1,529	36	624	3,958	91	605	3,934	55
Total	4,956	83,846	5,172.92	4,707	112,409	4,381.92	4,572	122,666	5,038.37

Source: HCD validated report 2014 (HCDA, 2014).

2.4 Varieties of French beans grown in Kenya for export.

Obtaining high yield and the right quality of pods with minimum production cost is critical to the farmer. Therefore selection of appropriate variety with high yield production, right quality of pods as per the market requirements and disease resistance attributes is an important step to every farmer. Different varieties of French beans respond differently to the climate and soil conditions (Kenneth, 2012) in terms of yield and quality of pods. The major French beans cultivars grown in Kenya are; Samantha, Teresa, Amy, Serengeti, Julia, Paulista and Monai (Ndegwa *et al*., 2010). These varieties are characterized by high yield, long picking duration and are resistance to pests and diseases.

In terms of economic importance, the French bean is ranked first among vegetables produced for the export market in Kenya and accounts for a significant proportion of total horticultural exports (HCDA, 2009). In 2009 French beans accounted for 60% of all exported vegetables and 21% of horticultural exports (HCDA, 2010), thus contributing significantly to employment of youth and women in the rural areas. Therefore, French bean growing is of importance in the socio-economic systems and livelihoods in Kenya. French beans in Kenya are mainly grown by small scale farmers, purely for export as a source of family income (Monda *et al.,* 2003). The major markets for Kenyan French bean is in the European Union (EU). In 2011, French beans accounted for 29% (about Ksh. 4 billion) of Kenya's total earning from vegetable exports of Ksh. 13.7 billion (Netherland Development Organization (SNV), 2012).

Despite the geographical position of Kenya and the favorable climate for growing French beans; the management of diseases, pests, and pesticide residues are a challenge. MRLs are set to minimise exposure of consumer to pesticide residue, protect human health and facilitate trade both locally and internationally. Noncompliance of French beans with MRLs as set out in EU regulation 369/2004 leads to loss in income in the value chain due to rejection and penalties. Small scale farmers in Kenya have witnessed increased production of French beans leading to good economic income (Table 2.1)

2.5 Fungal diseases affecting beans with pods in Kenya.

Diseases caused by bacteria or fungi, if not managed at the right time using the correct methods can lead to 100% loss of a crop. The use of agrochemicals to control diseases in French bean has increased due to numerous fungal diseases found in Kenya. Angular leaf spot, Powdery mildew, Anthracnose, Fusarium root rots, Rust and Downy mildew are the fungal diseases encountered by exporters interviewed in French bean production (Table 2B, Appendix 1). Carbendazim is a fungicide registered in Kenya for the control of Anthracnose and Angular leaf spot (PCPB, 2015).

2.5.1 Anthracnose.

Anthracnose (Figure 2.2) occurs in many regions of the world and is a major disease of the common snap bean (Phaseolus vulgaris L); can also occur in other legumes (Bush, 2014). It is caused by the *Colletotrichum lindemuthianum* (Hagedorn & Inglis, 1986), fungus. The fungus survives from season to season on infected plant debris and seeds (Hagedorn & Inglis, 1986), it affects all parts of the plant above the ground which includes leaves, petioles and pods. In beans with pods, the diseases noticeable symptom is irregular or sunken lesions on bean pods. Severely diseased plants are greatly reduced in vigor and yield. The disease can lead up to 100% crops loss if not managed. Anthracnose is favored by cool temperature (Hagedorn & Inglis, 1986) and is spread by splashing of spores by rain or irrigation water. The disease can also be spread by cultivars, animals and human; the disease can be managed by;

i) Use of clean seeds
ii) Use of resistance cultivars
iii) Practice crop rotation 2-3 years and
iv) Use of foliar fungicides**.**

Figure 2.2: Anthracnose lesions on French bean and enlarged view of tan to pink spore masses Bush, 2014)

2.5.2 Angular leaf spot.

Angular leaf spot is a disease of the tropics and sub-tropics that is caused by the fungus *Phaeoisariopsis griseola* (Bergamin, 1997). The disease is found in beans in many regions of the world (Hagedorn & Inglis, 1986). It affects all parts of the plant above the ground which includes the stem, leaves, petioles and pods. Symptoms are evident generally on the leaves at late flowering or early pod formation. They are characterized by brown or red angular spots (Figure 2.3). Leaves may also fall prematurely. Severely diseased plants have reduced vigor and poor yield (Hagedorn & Inglis, 1986). A humid condition favors the disease development and is spread mostly by wind-blown spores.

The disease can be managed by:

i) Use of clean certified seeds.
ii) 2 years crop rotation
iii) Burying previously infected beans debris underground
iv) Use of fungicides such as carbendazim to protect beans foliage and pods.

Figure 2.3: Angular leaf spot lesions on the underside of French bean leaf (Hagedorn & Inglis, 1986).

2.5.3 Powdery mildew.

The disease occurs worldwide in beans and is caused by the fungus *Erysiphe polygoni*, but does not cause significant damage as other fungi diseases (Hagedorn & Inglis, 1986). The fungus is spread through spores that are easily dispersed from one area to another by rain, wind and insects (Hagedorn & Inglis, 1986). The disease can also be spread by seed. It is characterized by formation of faint dark spots on the leaves. Affected leaves become dwarfed, turn yellow and fall down (Figure 2.4). In pods it forms small moist looking spots which develop into white powdery masses (Hagedorn & Inglis, 1986).

The disease can be managed by:

i) Use of clean certified seeds.

ii) Uses of pesticides early before the pods are affected

Figure 2.4: A photo of French bean leaves affected by Powdery mildew (Hagedorn & Inglis, 1986).

2.5.4 Fusarium root rot.

Fusarium root rot is a soil borne disease which is found in almost all soils. The disease is caused by the fungus *Fusarium solani f. sp.phaseoli*. The infections vary since nearly all plants have some degree

of root rot. The causal organism can survive in the soil for long and can be spread by movement of soil (Kenneth, 2014). Lack of crop rotation can lead to build up of the pathogen in the soil. However the impact of the disease is insignificant where the crop is strong and vigorous. The small roots rot away while the tap root will develop a reddish brown or dark brown discoloration (Figure 2.5).

The disease can be managed by:

i) Practice of crop rotation.

ii) Destroying bean refuse.

iii) Plant in well drained and fertilized soil.

iv) Use of treated seeds.

Figure 2.5: French bean root affected by Fusarium root rot (Kenneth, 2014).

2.5.5 Rust.

It has a significant economic importance in dry beans, however it can also affect French beans. Rust is caused by the fungus *Uromyces appendiculatus* (Kenneth, 2014). It affects the underside of the leaves, pods and the stem by forming tiny, almost white, pustules that later become distinct and

reddish-brown (Kenneth, 2014). The disease mainly affects the leaves and is less in stems and pods (Figure 2.6). Heavily infected leaves fall off the plant.

The disease can be managed by;

i) Practice of crop rotation.
ii) Planting fungi treated seeds.
iii) Use of resistance varieties.
iv) Spraying fungicide when they are spotted

Figure 2.6: A Photo showing a French bean leaf affected by Rust (Kenneth, 2014).

2.5.6 Downy mildew.

Downy mildew is caused by the fungus *Phytophthora phaserioli*. The disease mainly affects the pods. Infected pods shrivel, die, and turn black, and often remain attached to the plant (Edward *et al.*, 2014). However, the disease can also affect young shoots, flowers, and leaves (Figure 2.7). Nutrient stressed crops are susceptible to the disease when the weather is favorable. Infection by Downy mildew can destroy crops in a few days under favorable conditions. The fungal spores are easily dispersed by wind or splashing rain drops.

The disease can be managed by;

i) Use of resistance varieties.
ii) Practice of crop rotation.
iii) Avoiding use of seed of a previously affected crop.
iv) Spraying infected crop with fungicide**.**

Figure 2.7: The white dense cottony-like mycelia is a sign of invasion of the beans by *Phytophythora phoserioli* (Andrew *et al.,* n.d)

2.6 Carbendazim.

Fungicides are chemical compounds or biological organisms used to kill or inhibit growth of fungi or fungal spores. Carbendazim (methyl benzimidazol-2-yl Carbonate (IUPAC) is a systemic benzimidazole fungicide with protective and curative action (Australian Pesticide and Veterinary Medicine Authority, 2009). It is used for the control of diseases caused by a broad range of fungi such as Angular leaf spot, Powdery mildew, Scorch, Fusarium root rot and blight, affecting vegetables, fruits and field crops. Carbendazim is both a breakdown product of benomyl and thiophanate-methyl fungicides in plants and the environment (Alicja, 2010). The structure of carbendazim is shown in Figure 2.8.

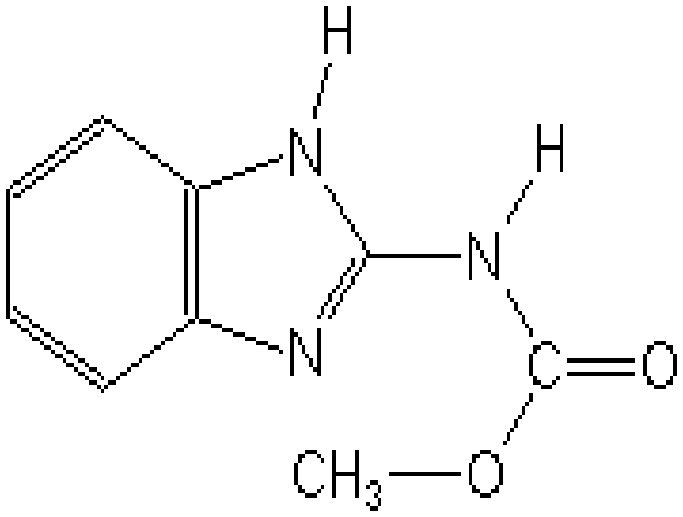

Figure 2.8: The chemical structure of carbendazim (IUPAC).

2.6.1 Use of carbendazim.

Carbendazim is used for the control of a broad range of fungal diseases in vegetables; fruits and field crops. It is used for foliar application, post-harvest food storage and seed dressing (Australian Pesticide and Veterinary Medicine Authority, 2009). The mode of action of Carbendazim involves absorption through the root and green tissues of the plant. It kills the germinating spores of the fungi and prevents the outbreak of disease, referred to as preventive action (Cambodia Harvest, 2012). When applied after the infection has already started, it attacks the developing mycelium and arrests its spreading by suppression of sporulation. This is referred to as curative action. In Kenya commercial carbendazim based formulations are available as a single molecule or combined with other molecules such as Mancozeb, Imidacloprid, Metalaxyl, Iprodione and Permethrin.

2.6.2 Health effects of carbendazim.

Carbendazim is classified by the World Health Organization (WHO) as 'unlikely to present hazard in normal use (WHO, 1999). The primary source of carbendazim exposure for the general human population is dietary intake (PAN, 2014). Carbendazim has been in the news because of its residues in food stuff. According to EU Food Authority, carbendazim had the highest detection in fruits and vegetables in 2011 which stood at 5.8% of laboratory tests performed (Pesticide Action Network Europe (PAN), 2014).

The acute oral LD50 (dose at which half the sample is dead) for rats is >15000 mg/kg and >2500 mg/kg for dogs (Tomlin, 2000). For agricultural workers, occupational exposure during manufacture or use is considered to be within acceptable levels. According to International Programme for Chemical Safety (IPCS) there is no danger of exposure to carbendazim during its production and use

in agricultural production (Pesticides News, 2002). In terms of chronic toxicity, carbendazim is a suspected endocrine disruptor (Friends of the earth, 2001). It has been included by the European Commission on a priority list of chemicals that are believed to affect hormone function (Pesticides News, 2002). Carbendazim is therefore considered to have an effect on human reproductive system and is also capable of causing malfunctions in the foetus at very low doses (Pesticide Action Network Europe, 2014).

Though carbendazim is not known to course mitosis, it affects the chromosome and thus causes infertility in men (Pesticide Action Network Europe, 2014). Recently researchers testing the effect of carbendazim on cultured human lymphocytes concluded that carbendazim is a potent aneugen (affects the number of chromosomes) even at low exposures (Mahmood *et al.*, 2001). In terms of wild life and environmental effect, carbendazim is highly absorbed in soil organic matter and sediments. Therefore animals or organisms living in sediments are likely to be exposed to carbendazim e.g. earth worms and fish (WHO/FAO, 1996). Carbendazim is very dangerous to aquatic organisms and even a buffer zone of 20 meter towards water bodies is not protective enough (Pesticide Action Network Europe, 2014).

2.7 Food safety and pesticide residue.

There is increased demand for safe food all over the world. This has been due to increased public awareness on the impact on human health associated with exposure to contaminated food. Food contaminants are harmful substances which are either biological or chemicals substances which enter the food chain through the environment or chemicals used for pest control during the active growth of the crop, transportation, storage and processing. Because of the health concerns and consumer demands for safe food; national, regional, international and private guidelines have been established fundamentally to ensure controls and conformance in the production, transport, storage and processing of food (Codex Alimentarius Commission (CAC), 2009). The overall goal is to provide assurance that the food meets the requirements of the consumer and is safe to the consumer and environmental health.

2.8 Pesticides.

A pesticide is a substance that has the ability to control pest or disease. On the other hand a pest is a plant or animal that can cause harm to human or the environment (Pesticide compliance & safer use principles, 2010). Increase in human population, development of technology and climate change has caused;

i) High demand of food.
ii) Change in pest patterns.
iii) Demand for quality food.

Pesticide include insecticides, herbicides and fungicides. Pesticides are used to prevent or reduces crop loss due to pests, improves crop yield, maintain quality and protect human health from food borne diseases. Pesticide carried over to the food is referred to as pesticide residue and exposes the consumer to the dangers of the chemical. Some of the pesticides have acute or chronic effect to the consumer exposed. To protect the consumers, national and international levels have been established for various commodities and molecules. The Maximum Residue Levels (MRLs) is the upper limit of pesticide residues concentration legally accepted in or on food and feed based on good agricultural practices to ensure the minimum exposure to the consumer and environment (EU Regulation, 2005).

2.8.1 Setting of maximum pesticide residues limits in the European Union.

Worldwide MRLs are regulatory standards that help to monitor whether an agricultural or veterinary chemical has been used as directed on an approved label. MRLs exceedene is an indicator of misuse of the chemical but does not normally mean there is a public health or safety concern. According to Europe Union Regulation (EC) No. 178/2004, Food Safety Authority is the body charged with the responsibility to develop and implementation of regulation on food.

Regulation (EC) No 396/2005 (Figure 1A, Appendix 1) on maximum residue levels of pesticides in or on food and feed of plant and animal origin is the EU guiding principle for setting up MRLs in various plant produce. The main objective is to give a high and consistent level of consumer protection and harmonized MRLs across Europe.

2.8.2 Compliance of French beans from Kenya with EU requirements.

From 2013 the European Union countries have been closely monitoring the incidence of pesticide residues in peas and French beans imported from Kenya. EU regulations specify the levels of pesticide residues permitted in different foodstuffs, and where these levels are exceeded, action is taken. From 2011, pesticide residues have been detected in a number of consignments imported from Kenya. As a result, the European Commission increased the intensity of border controls of peas and beans imports from Kenya to 10% sampling from January 2013 for pesticide residues screening before entry to EU (EU Regulation 669/2009). Between January 2013 and November 2014, Kenya received a total of 103 border rejection notifications, with 56 occurring in 2013 and 47 within January to November 2014 (EU Regulation, 2009).

The satisfactory level of compliance reported in 2015 for French beans consignments resulted to de-listing as observed in Commission Implementing Regulation (EU) 2015/1012 of 23 June 2015 L 162. The measures taken by the country includes; strengthening of the National pesticide residue testing laboratory, labelling of plant protection products and awareness creation.

2.8.3. Impact of non-compliance to Maximum Residue Limits (MRLs).

The EU increased controls have impacted negatively on the vegetable sector. Since French beans are consumed fresh, they have a very short shelf-life. The time taken at each step from harvest to the table is therefore crucial for the beans to be consumed fresh. The controls led to a delay which is translated to a reduced sell-by date. This situation has direct economic impact on the lives of thousands of farmers and workers in Kenya, where agriculture is the major contributor to socio-economic welfare. A research was conducted to assess the impact of the controls and the findings showed a big decline in export volumes to the EU in January to March 2013 compared to the same period in 2012 (USAID-KAVES, 2015). There was also a reduction in the number of pack-house workers, and reduced sourcing of French beans from small-scale out-growers by exporters, who have traditionally been major producers of these crops. Kenyan farmers and stakeholders could only hope that the efforts made by the government of Kenya were good enough to convince the European Union Commission delegation to give Kenya a clean bill of health and consequently have the increased inspection lifted once and for all.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area.

Two trials were conducted at selected sites, Kambaa in Machakos and Naivasha in Nakuru counties. These sites represent major French beans growing areas in Kenya for export. The French beans were planted in September, 2015 and March, 2016 to represent short and long rain seasons respectively French beans were grown at optimum temperature of 20^0C -30^0C and altitude of 1200 to 2100 meters above sea level in a well distributed medium to high annual rainfall of about 900 to 1,200mm per year. However, to ensure continuous production throughout the year, supplementary irrigation of up to 50mm of water was applied per week in the areas where rainfall was not adequate.

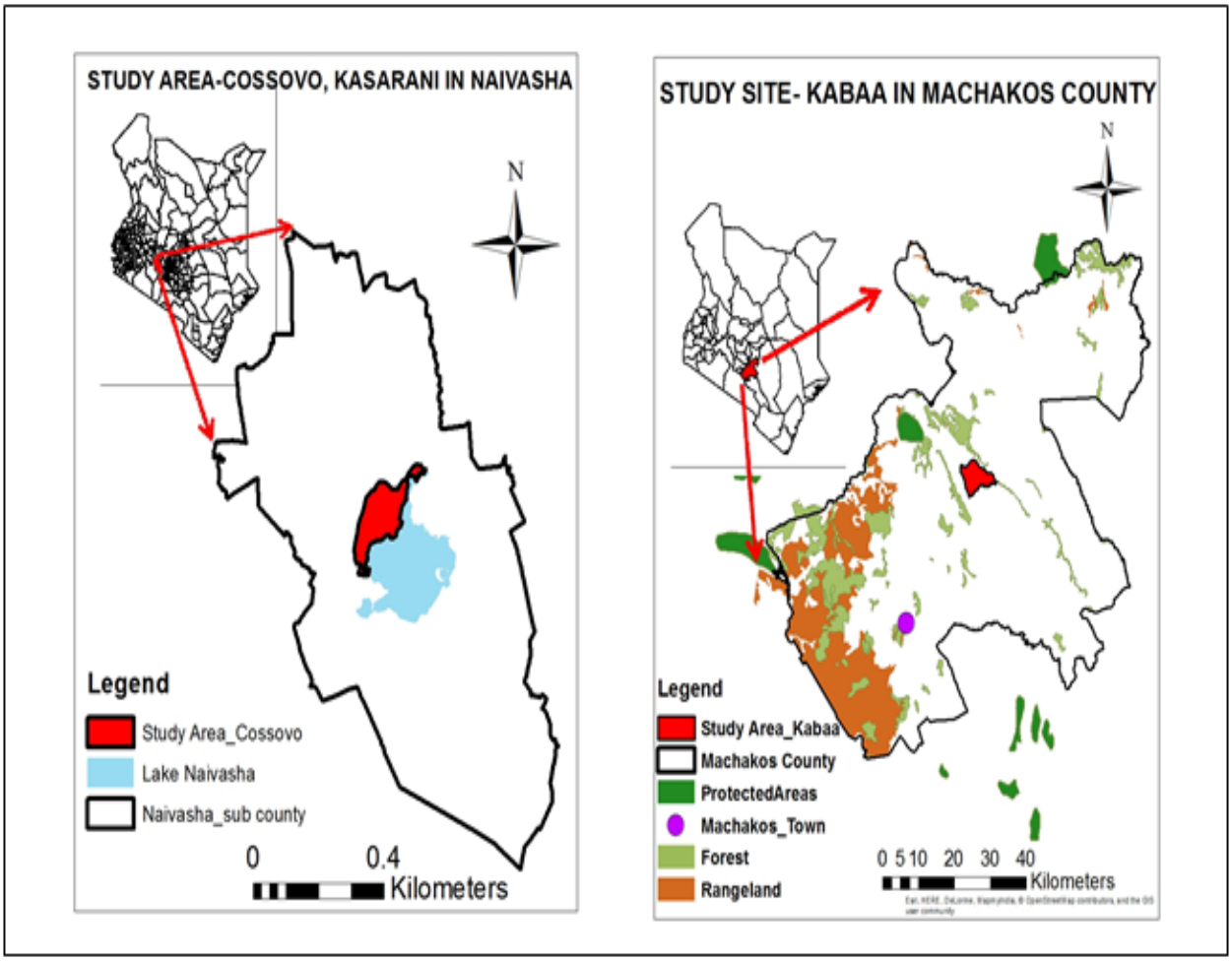

Figure 3.1: Map of Kenya showing location of the trial sites.

3.1.1 Kambaa site.

Kitwamba farm with a total area of 8.5 Ha registered under Sacco fresh exporters located in Kambaa Machakos County was used. Kambaa trial site lies between latitude of 0010 14.4' S, longitude of 0370 28.5' E at an altitude of 1,268 m above sea.

3.1.2 Naivasha site.

Mahe farm with a total area of 15 Ha registered under Wilham exporters in Naivasha Nakuru County was used. The trial site lies between, latitude of 000 45' S, Longitude 0360 17.7' E at an altitude of 1,920 m above sea level.

3.2 Selecting French beans Variety and Carbendazim formulation.

Questionnaires (Table 2A, appendix 2) were distributed to 20 exporters to identify French beans' variety commonly grown and the carbendazim formulations in the two study areas. Based on the exporters' respondents', Serengeti French beans variety, Chariot 500 SC and Rodazim SC carbendazim formulations were selected.

The list of registered pesticides in Kenya according to the 9th Edition of Pest Control Products Board (PCPB, 2015) was used in ensuring that the selected carbendazim formulations were registered for foliar use in Kenya in French beans.

3.3 Layout of the field trial plots.

The field trial was set up in plots of 13 m by 10 m (130m^2) for every treatment separately, giving a total area of 260m^2. All the treatments were set in three replicates. The plots were divided into 4 equal blocks of 2 m x 10m (20m^2), three replicate treatments and one control (FAO, 1986) were used. Adequate buffer zone of 1 m was left in between each of the block and around the plot (Figure 3.4) to prevent contamination due to spray drift. The plot and blocks were also uniquely identified with permanent labels containing field identification number, planting date, expected harvesting date and the variety planted.

3.4 Site preparation.

The trial sites that had previously been used to plant French beans were ploughed and harrowed by tractor. The sites were divided into blocks of 2m by 10 m separated by 1 m foot path. 2 ridges of 70 cm wide and about 30 cm high were formed manually in every block (Figure 3.2). The blocks were labeled as KT1a, KT1b, KT1c and KC1 for the first carbendazim formulation Chariot 500 SC (Regime 1) and KT2a, KT2b, KT2c and KC2 for the second formulation Rodazim SC (Regime 2).

Figure 3.2: A block with ridges prepared before planting.

The crops were planted in two double rows with 35cm space between the double rows and 15cm space within the double rows (Figure 3.3).

Figure 3.3: French beans planted in double rows.

3.5 Formulation.

The carbendazim formulations were purchased from licensed agrochemical retail shops in Kambaa and Naivasha for use in Kitwamba and Mahe farms respectively. The formulations were stored in a controlled environment before use.

3.6 Pesticide application.

The formulations were applied in a manner that represents the common application technique used by commercial growers, while following the directions specified by the manufactures (Table 3.1). A pesticide application knapsack spray equipment at each site was evaluated for performance before use to ensure uniform application of the test solution for adequate canopy penetration and coverage

Table 3.1: Rate of carbendazim pesticide application.

Trade name	**PCPB Reg. No.**	**Weight (g)**	**Treatment**	**Dosage**	**Recommended rate**
Chariot 500 SC	PCPB (CR) 1084	500	Regime 1	20ml/20l	0.5l-1.0l/ha
Rodazim SC	PCPB (CR) 0378	500	Regime 2	20ml/20l	0.75l/ha

3.6.1 Calibration of spray equipment.

The pesticide formulation was applied using knapsack spray equipment similar to that used by the farmers (Figure 3.6). The equipment performance was evaluated by carrying out the following tests;

- Flow rate (Nozzle output)
- Speed (operator speed)
- Rate of application

Calibration of the pesticide application equipment was conducted before the first application of the pesticide using the performance evaluation parameters.

Figure 3.4: Knapsack spray equipment used for pesticides application.

3.6.2 Rate of application

The treatment was applied to the test crop at the rate of 20ml per 20 litres of water following the manufacturer's instructions (Table 3.1). The application was done once when the pods were ready to harvest. The amount applied was 10 mls pesticide in 10 litres of water in 0.008 Ha.

3.6.3 Supplementary crop treatments

Apart from the normal agronomic practices no additional pesticides was used to control pests in test plots during the experiment. Foliar feed fertilizers were used in both the control and treatment plots.

3.7 Sampling.

The experiment was conducted concurrently at both regions and sampling done in the month of September and December, 2015 for short rain season (season 1); March and May, 2016 for long rain season (season 2). French bean pods samples were collected for analysis in season 1 and 2.

3.7.1 Treated and control plots.

Sampling was carried out as per the schedule of days (d) related to the harvest (H) and analysis done (X) on the sample (Table 3.2). One (1) kg of beans pods sample ready for harvest were taken at 0, 3, 7, 14 and 16 days after pesticides application for the treated blocks. At day zero the samples were

collected when the crop was dry to avoid contamination of the person taking the sample or cross contamination of samples.

Proper sample handling practices were observed i.e. use of clean gloves when harvesting to prevent transfer of pesticide residue from one sample to another or removal of pesticide on the surface was be observed.

Table 3.2: Sampling points for treated blocks in days.

Treatment	Treatment 1 –Plot 1,2 and 3				
	H1	H2	H3	H4	H5
Sampling frequency	X (0 d. PHI)	X (3 d. PHI)	X (7 d. PHI)	X (14 d. PHI)	X (16 d. PHI)

Two samples of 1kg each of beans pods ready to harvest were taken at 0, 3, 7, 14 and 16 days from the control plot (Plot 4) before sampling from the treated plots (Plot 1,2 and 3). Sampling was carried out as per the schedule of days (d) related to the harvest (H) (Table 3.3). No pesticide containing carbendazim or its metabolite was applied on the control plot.

Table 3.3: Sampling points for control blocks in days.

Control	Control plot 4				
	H1	H2	H3	H4	H5
No application done	X (0 d.PHI)	X (3 d. PHI)	X (7 d. PHI)	X (14 d. PHI)	X (16 d. PHI)

3.8 Sample packaging and transportation to the laboratory.

The bean samples were packaged in a double polythene bag, uniquely labeled in order to maintain the integrity and traceability of the sample. The packaged samples were immediately placed in a polyethylene cool box with dry ice and transported in the frozen form to the laboratory. The samples were not allowed to thaw, either before or during transportation.

On arriving in the laboratory Verification of the integrity of the packaging, sample code and the number of samples was done. The frozen samples were stored at -18^{0}C when processing or analysis was not done on the same day to prevent degradation of the pesticide. Samples treatments, harvesting and transportation to the laboratory were carried out respectively in both the short and long rain seasons as explained in section 3.7 and 3.8.

3.9 Sample analysis.

The samples were analysed using KEPHIS laboratory accreditated QuCHERS (Quick, Cheap, Efficient, Robust and Safe) multi-residue method of analysis for pesticide residues in fruits and vegetable. To determine the concentration of carbendazim in the sample Liquid Chromatography technique with a triple quadruple mass detector (LCMSMS- Agilent 6430) method was used. The method involved processing, extraction, clean-up and analysis of the final fraction for analysis by Liquid Chromatography.

3.9.1 Sample processing

The frozen samples were cut into small particle size of less than 2mm using Stephan chopper and homogenized by warring blender. Each sample was sub-divided into two analytical portions of 50g. The retained samples were kept frozen at -18^{0}C until the end of the study.

3.9.2 Pesticide extraction.

Weighing was done using a calibrated analytical balance (Adams 210). The balance was checked to ensure that it was in good working condition and the calibration verified using traceable masses (SI). 10g ±0.1g of the homogenized analytical portion and 5 control samples were weighed by difference into different 50ml single use extraction polyethylene tubes. One of the control samples was fortified with 50 µl of carbendazim standard solution to achieve a spiking level of 0.05µg/g. 50 µl of procedural internal standards Dimethoate D6 (10mg/kg) was added to the samples, control samples, spike sample and solvent blank (Acetonitrile) to achieve, 0.05µg/g final concentration.

10ml±0.2ml of acetonitrile was added into sample, control samples, spike sample (recovery) and solvent blank; the tube was tightly closed and shaken vigorously by hand. 6.5 g of premixed extraction

salt (4g±0.2g Magnesium sulphate anhydrous, 1g±0.05g sodium chloride, 1g±0.05g Trisodium citrate dehydrate and 0.5g±0.03g disodium hydrogen citrate sesquihydrate) was added to the mixture and vortexed (Wisemix-VM-10) for 1 minute ± 10 second to disperse the sample into the solvent. Finally the mixture was centrifuged (Universal 320 R) for 5 minutes with centrifuge set at 3700rpm.

3.9.3 Sample clean up and analysis.

6ml aliquot of each organic phase of the extracts was transferred into a single use polyethylene centrifuge tube containing 1.05g of pre-mixed clean-up salts (0.15g primary secondary amines (PSA) and 0.9g anhydrous magnesium sulphate). The tube was vigorously shaken by hand to avoid caking and vortexed for 1 minute ± 10 seconds and centrifuged for 5minutes with the centrifuge set at 3700 rpm. 4 ml±0.2ml of the extract was filtered through a 0.45µm membrane filter and transferred into centrifuge tubes. 40 µl of 5% formic acid solution in acetonitrile (v/v) was immediately added to the extracts to adjust the pH to approximately pH 5.

To 2mls of the extracts, 20 µl of formic acid (10 µl /ml of sample) and 60 µl of D-sorbitol (30 µl /ml of sample) was added and vortexed to mix .To 500 µl of the mixture add 500 µl of HPLC grade water was added and vortexed for 1 minute ± 10 seconds to mix. From the final mixture (concentration 1g/ml) were transferred to a 2ml sample vial, capped and taken to the LCMSMS for analysis.

To ensure quality control, certified reference standard, control samples, spike (recovery), solvent blank, calibration and internal standard were used to monitor the performance of the procedure and instrument during the analysis. Quality control samples were prepared and analysed together with the analytical samples. The flow chart is summarized in Figure 3.7

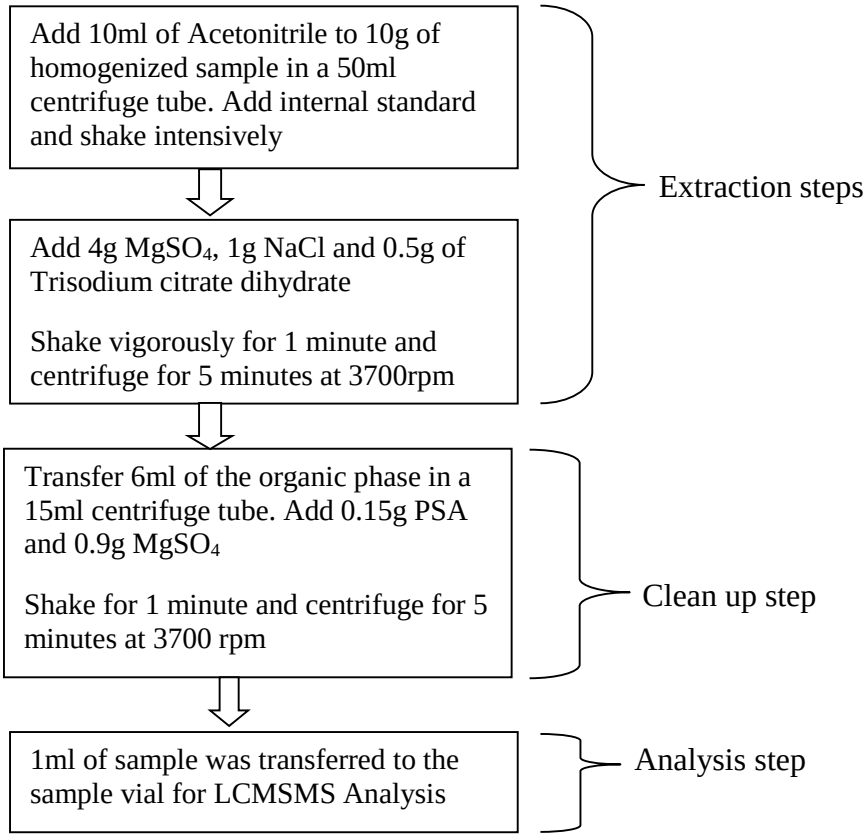

Figure 3.5: Flow chart for sample analysis.

3.10 Calibration curve standards preparation.

Calibration standards were prepared at concentrations of 0.01, 0.025, 0.05, 0.075 0.1 and 0.2 µg/ml for validation of method (Table 3.4); 0.01, 0.05 and 0.2 µg/ml for routine analysis using the extract.

Table 3.4: Calibration curve standard preparation.

Concentration level	1000µg/kg carbendazim standard solution	Sample extract	Solvent (water and acetonitrile, 80:20)	Total volume
10µg/kg	10µl	500µl	490µl	1000µl
25µg/kg	25µl	500µl	475µl	1000µl
50µg/kg	50µl	500µl	450µl	1000µl
75µg/kg,	75µl	500µl	425µl	1000µl
100µg/kg	100µl	500µl	400 µl	1000µl
200µg/kg	200µl	500µl	0 µl	1000µl

3.10.1 Detection limit for carbendazim.

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. The LOD of carbendazim was expressed as the analyte concentration required to produce a signal greater than three times the standard deviation of the noise level as determined by empirical approach consisting of measuring progressively more dilute concentrations of analyte and employing the following relationship:

$$LOD = \frac{3x \text{ Noise peak area x concentration of standard injected (ng/ml)}}{\text{Analyte response in the lowest calibration point}}$$

3.11 Statistical data analysis.

Correlation of pesticide residues with physicochemical parameters was done using Pearson's correlation. Pearson, r is a measure of linear dependence between two variables X and Y giving a value of +1 and -1 inclusive. Where 1 is a total positive correlation, 0 is no correlation and -1 is negative correlation. Positive value indicates that the changes are in the same direction while negative values indicate inverse variation relationships. Correlations coefficients above 0.5 are considered to be strong while below 0.5 is considered to be weak. The significance of correlations is indicated by the P value. Correlations is significant if $p<0.05$ and is not significant if $p>0.05$ (APA, 2001).

The concentration of carbendazim obtained from the calibration curve was calculated using the following formula;

$$\text{Concentration in mg/kg} = \frac{[\text{C sample x Final volume of extract (ml)}]}{\text{Sample weight (g)}} \quad \text{.....Equation 3.1}$$

Where C sample is the amount of pesticides in mg/kg, read from the calibration curve.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Variety and formulation selection.

A questionnaire was administered to obtain information from exporters on; the common variety of French beans grown for EU export market and commercially available carbendazim pesticide formulations registered in Kenya for use in control of major fungi diseases in French beans (Figure 2A, Appendix 2).The survey revealed that exporters deals with exports of more than one variety of French beans depending on the client specifications or the targeted market requirements (Figure 2B, Appendix 2). Other factors which determine the variety includes resistance to pest, disease and drought. All the exporters interviewed had valid export licensed and sourced their produce from contracted farmers (Figure 2A, Appendix 2).

There were ten varieties of French beans grown by the exporters interviewed for export to the EU market. 80% of the respondents interviewed grow Serengeti variety for exporters as shown in Figure 4.1. Since Serengeti French beans variety was the most preferred for export to EU market, it was selected for the study.

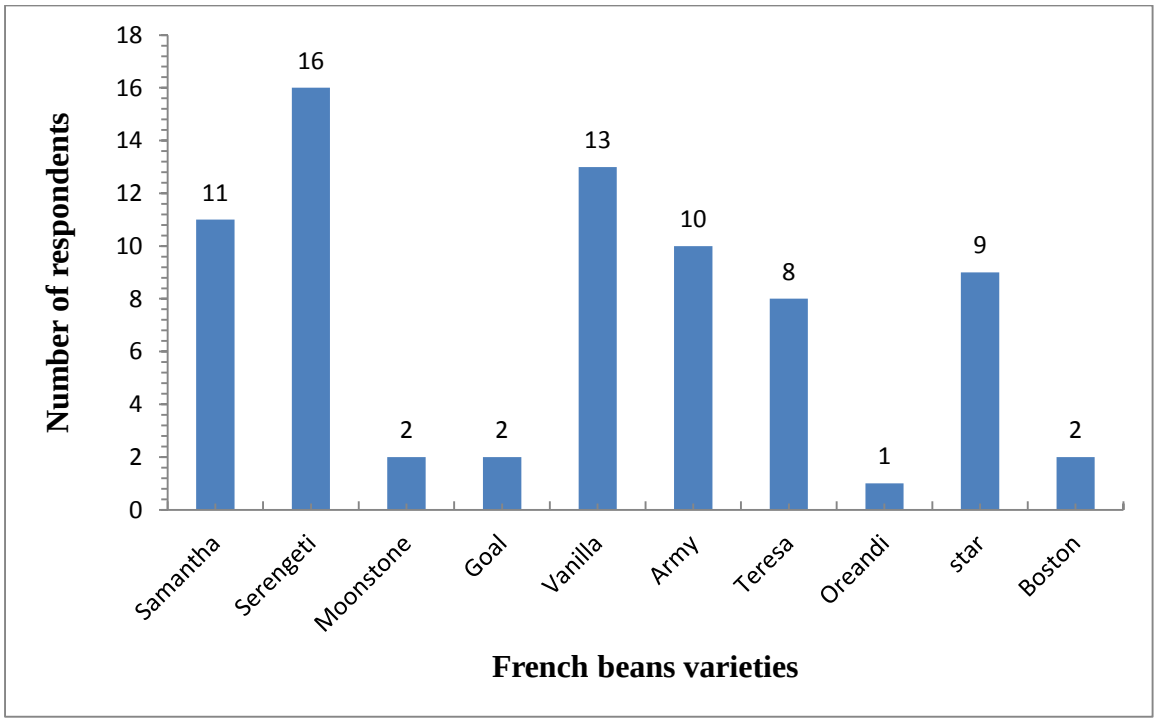

Figure 4.1: Graph showing the French beans varieties grown by the respondents.

The questionnaire was also used to collect data on the common fungi varieties affecting French bean crop as per the data collected, all the respondents interviewed were aware of fungal diseases affecting French beans and they were using pesticides to protect the crop against the diseases damages (Figure 2B, Appendix 2). It was established that exporters use approved policies and procedures to ensure safe use of plant protection products and disposal of pesticides wastes. Technical persons employed by the exporters were responsible to; identify pest and diseases, advice the farmers on selection, and application of plant protection products. The most common fungal diseases experienced by farmers growing French beans for export are Anthracnose, Rust, Blight, Angular leaf spot, Powdery mildew, Ascochytae, Halo blight, Downy mildew, Fusarium wilt, Bacteria wilt and Dumping off. 80% of the respondents indicated that they experienced rust disease in French beans, Figure 4.2.

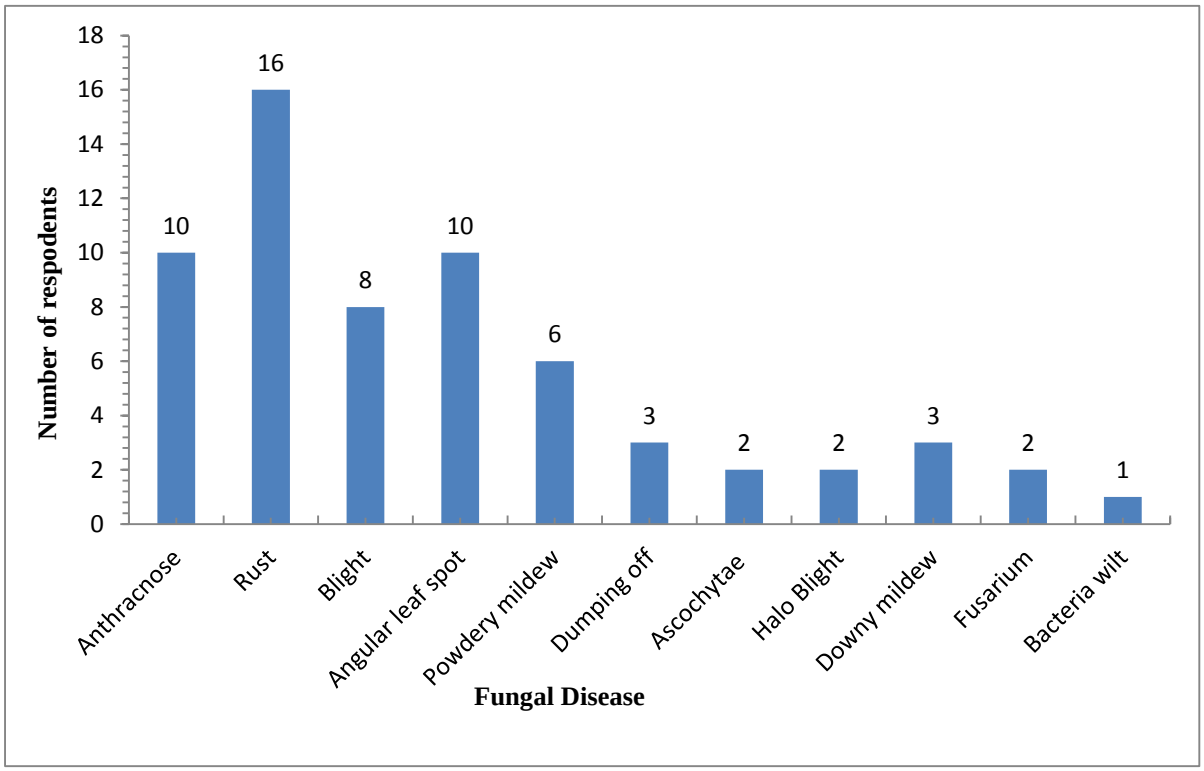

Figure 4.2: Graph of fungal diseases affecting French beans as obtained from the respondents.

The survey indicated that Thiomethoxam, Matalaxyl, copper, Sulphur, Tebuconazole, Azoxystrobin, Carbendazim, Difenoconazole and Potassium diphosphate are the common active molecules used to control fungal diseases by the respondents. Pesticide formulations based on these molecules are registered in Kenya and are commercially available in local agrochemical retailers. 27.8% of the exporters interviewed use formulations based Azoxystrobin while 26% use carbendazim as shown in Figure 4.3. The pesticides formulations selected for this study were those commonly used locally by farmers for the control of fungal disease in French beans (Chariot 500SC and Rodazim SC used by 80% of the respondent.

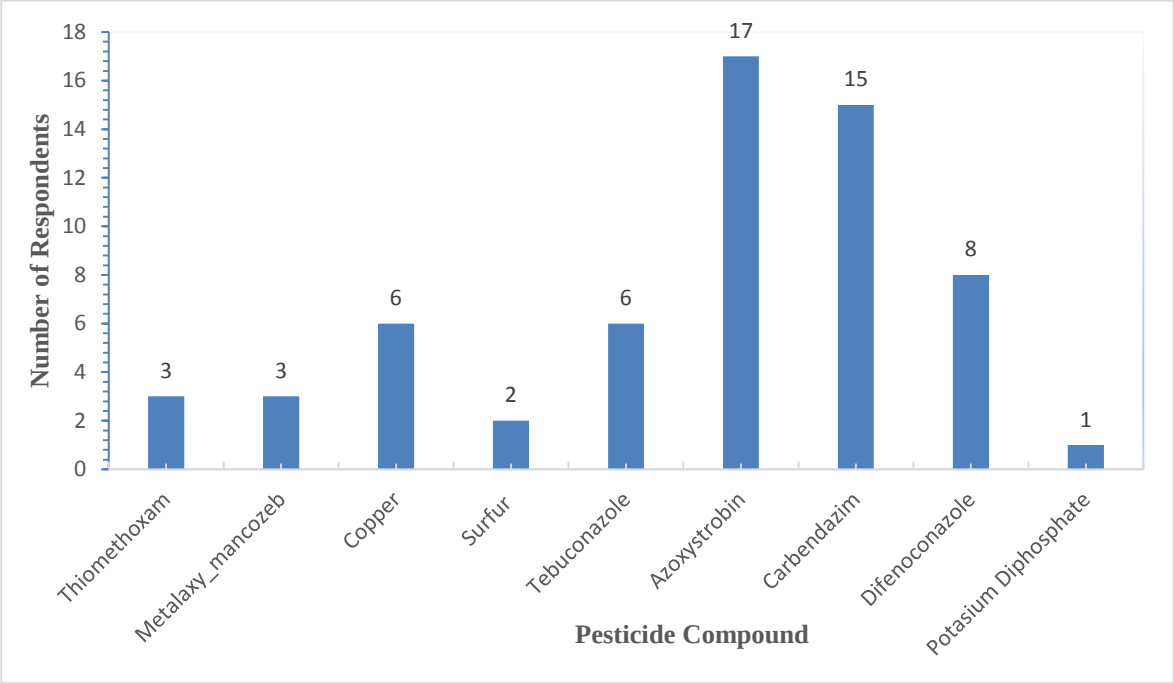

Figure 4.3: Graph summarizing the responses of exporters on the pesticide compounds used to control fungal diseases in French beans.

4.2 Quantification tests.

Quantification of the samples was based on calculations from carbendazim standard solutions (Prepared in 20% Acetonitrile) calibration curves in the concentration range of 10µg/kg to 200µg/kg (Figure 3A, Appendix 3). The standard calibration curve provided a straight line for carbendazim for the best line of fit obtained by plotting the relative response factor, that is the ratio of instrument response (peak area), against analyte concentration. The analyte lines gave a correlation factor (R^2) above 0.998 (Figure 3A, Appendix 3), indicating a high correlation between instrument response ratio and analytes concentration.

Sample analyte concentrations were obtained by interpolation from the graphs which applies the equation of the line;

$Y = mX + C$

Where Y= Peak area (Instrument response)

X=Analytes concentration,

m=Gradient, and

C= Constant.

4.3 Quality control of the method.

Quality control of the method was used to monitor the performance of the extraction and instrument system. Compliance with predetermined limits of the method during validation indicated that the method was under statistical control and vice versa. Carbendazim standard was fortified at a concentration of 50µg/kg into blank sample and Dimethoate D6 internal standard was spiked at concentration of 50µg/kg into all samples and standard. The recovery results were within the recommended range of 70% to 120% (Figure 5A, Appendix 5) and hence the values were not corrected for recovery (Hill, 1999).

The recovery was calculated from the equation;

$$\% x = \frac{C_o}{C_s}$$

Where: $\% x$ = % Recovery

C_o = Observed concentration

C_s =Spiked concentration.

The results indicated that the ratio of the qualifying ion (ion 205) abundance to that of the transition ion (ion 236.0) of Dimethoate D6 internal standard was 8.7 which is within the recommended range of 6.7 to 12.4 (Figure 6A, Appendix 6). The responses of the internal standard were also recorded in the control chart, a tool used to monitor the day to day performance of the method.

4.4 Test for outliers.

Grubbs' outlier test statistical tool in Microsoft excel was applied to determine whether any of the observations with minimum or maximum values in the replicate data sets were outliers. The data obtained from the LCMSMS (Table 8A, Appendix 8) were arranged in-order of the sampling points and test for any outliers using Grubbs test at the 95% confidence level. From the Grubbs' test results, none of the obtained data was found to be an outlier and therefore the data were not corrected for outliers (Table 7A, Appendix 7).

4.5 Dissipation of carbendazim pesticide residue in French beans for the two formulations.

The study was conducted to determine the dissipation of carbendazim in Serengeti French beans to establish a harmonized PHI. Tables 4.1A, 4.1B, 4.2A and 4.2B present the results on the dissipation of carbendazim in French beans with time for Chariot 500 SC and Rodazim SC respectively. Table

4.1A and 4.1B summarises data for the concentration of carbendazim residues result from application of Chariot 500 SC in Kambaa and Naivasha. Three days after application of the pesticide carbendazim residues were 496.2µg/kg and 349.0µg/k in Kambaa; 467.3µg/kg and 355.4µg/kg in Naivasha in short rains and long rains seasons respectively. The percentage degradation ranged from 46.0% to 60.6% giving a 14.6% difference between the lowest and the highest degradation. However it is observed that degradation was high in short rain than in long rains. This variation could be attributed to temperature. The percentage degradation in 7 days after application were between 68.8% and 82.5 % with reference to the initial carbendazim concentration. This gave a 13.7% difference between the lowest and the highest degradation. This shows that the degradation of carbendazim rate in the two season at 3 and 7 days was comparable. The progress on time after application of the pesticide resulted in a more dissipation of the residues. At 14 days after application the dissipation of Chariot 500 SC in the two seasons was between 96.7% and 100.00% (Table 4.1).

Table 4.1A: Summary of carbendazim residues detected in short rain season for Chariot 500SC

Short rain season		
	Mean Concentration (µg/kg)	
Days	Kambaa (µg/kg)	Naivasha (µg/kg)
0	1260.7 ±4	970.3 ±4
3	496.2 ±4	467.3 ±4
7	220.1 ±4	188.5 ±4
14	17.7 ±4	31.6 ±4
16	5.7 ±4	0.7 ±4
% Reduction by 3 day	60.6	51.8
% Reduction by 7 day	82.5	80.6
% Reduction by 14 day	98.6	96.7
% Reduction by 16 day	99.5	99.9

Table 4.1B: Summary of carbendazim residues detected in long rain season for Chariot 500SC

Long rain season		
	Mean Concentration (µg/kg)	
Days	Kambaa (µg/kg)	Naivasha (µg/kg)
0	645.9 ±4	675.2 ±4
3	349.0 ±4	355.4 ±4
7	127.9 ±4	210.9 ±4
14	1.5 ±4	0.0
16	0.0	0.0
% Reduction by 3 day	46.0	49.5
% Reduction by 7 day	72.1	68.8
% Reduction by 14 day	99.8	100.0
% Reduction by 16 day	100.0	100.0

Results in Table 4.2A and 4.2B shows that carbendazim pesticide residue levels 3 days after application of the pesticide were 492.8µg/kg and 455.8µg/k in Kambaa; 368.2µg/kg and 576.3µg/kg in Naivasha in short rains and long rains seasons respectively. The percentage degradation ranged from 36.4% to 52.6% giving a 16.2% difference between the lowest and the highest degradation. Similarly as observed in Chariot 500 SC the degradation was high in short rainy season than in long rainy season. The percentage degradation 7 days after application was between 75.1% and 80.2%. The progress of time after application of the pesticide resulted in more dissipation of the residues. At 14 days the dissipation of the Rodazim 50 SC in the two regions was between 97.7% and 100.00%.

Table 4.2A: Summary of carbendazim residues detected in short rain season for Rodazim SC.

Short rain season		
	Mean Concentration (µg/kg)	
Days	Kambaa (µg/kg)	Naivasha (µg/kg)
0	1039.4 ±4	678.4 ±4
3	492.8 ±4	368.2 ±4
7	244.1 ±4	134.5 ±4
14	3.9 ±4	15.7 ±4
16	0.8 ±4	0.6 ±4
% Reduction by 3 day	52.6	45.7
% Reduction by 7 day	76.5	80.2
% Reduction by 14 day	99.6	97.7
% Reduction by 16 day	99.9	99.9

Table 4.2B: Summary of carbendazim residues detected in long rain season for Rodazim SC.

Long rain season		
	Mean Concentration (µg/kg)	
Days	Kambaa (µg/kg)	Naivasha (µg/kg)
0	825.7 ±4	905.5 ±4
3	455.8 ±4	576.3 ±4
7	205.8 ±4	192.8 ±4
14	0.0	0.0
16	0.0	0.0
% Reduction by 3 day	44.8	36.4
% Reduction by 7 day	75.1	78.7
% Reduction by 14 day	100.0	100.0
% Reduction by 16 day	100.0	100.0

Tables 4.1A, 4.1B, 4.2A and 4.2B indicate that, the progress of time after application of the pesticide resulted in more dissipation of carbendazim (Chariot 500 SC and Rodazim 50 SC) used. The degradation was between 60% to 80% 7 days after application and 95% to 100.0% 14 day after application. 95% to 100% degradation at 14 days will lead to a realistic PHI in French bean considering their active growth period and time of harvesting for consumption.

The trends of dissipation of carbendazim for the two formulations used in the study during short and long rain seasons were compared by plotting the averages of the determined concentration of carbendazim in the samples at Kambaa and Naivasha against time. Figure 4.4A, 4.44B, 4.5A and 4.5B shows that the degradation of carbendazim in chariot 500 SC and Rodazim SC followed a similar pattern which is comparable in short and long rain seasons. According to EU regulation 396/2004, the determined concentration was below the maximum residue limit set at 200µg/kg 7 days after application as shown in Figure 4.4A, 4.4B, 4.5A and 4.5B.

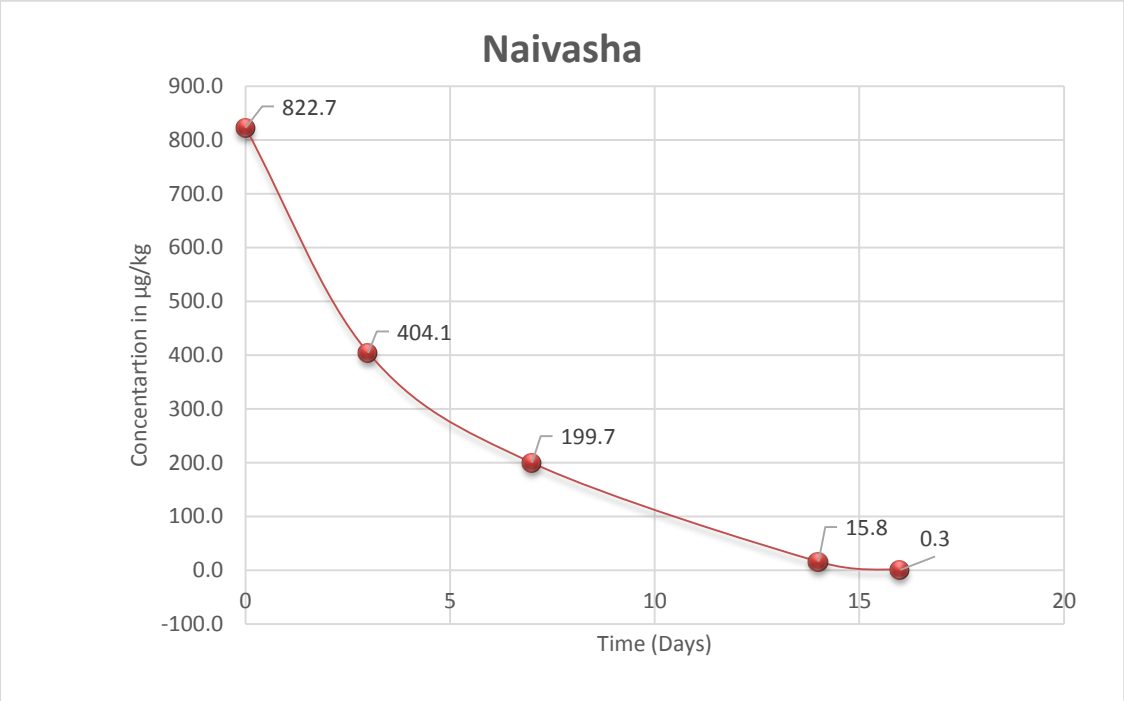

Figure 4.4A: Graph showing average reduction of carbendazim concentration against time for Naivasha Chariot 500 SC samples (Short and Long rain season).

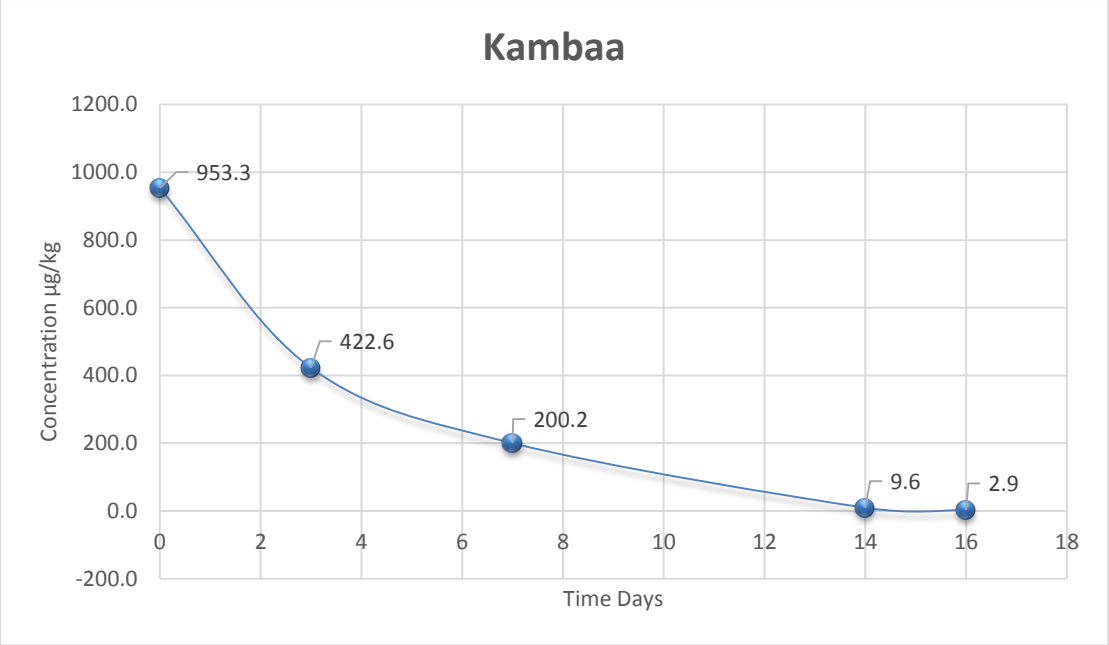

Figure 4.4B: Graph showing average reduction of carbendazim concentration against time for Kambaa Chariot 500 SC samples (Short and Long rain season). .

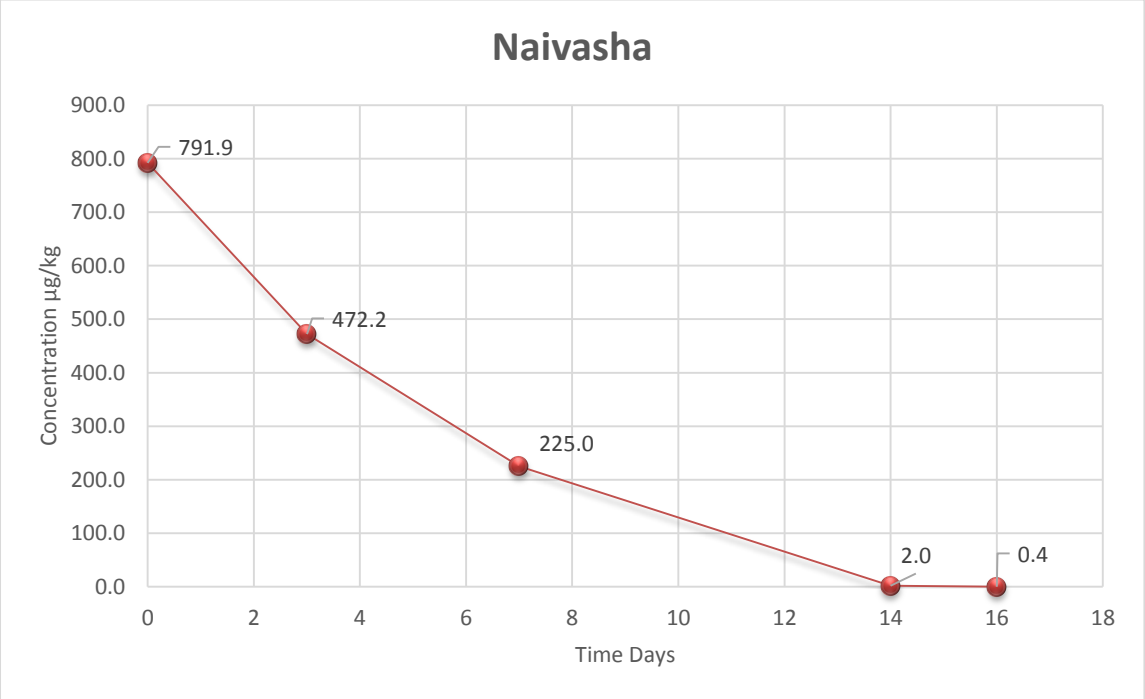

Figure 4.5A: Graph showing average reduction of carbendazim concentration against time for Kambaa Rodazim SC samples (Short and Long rain season). .

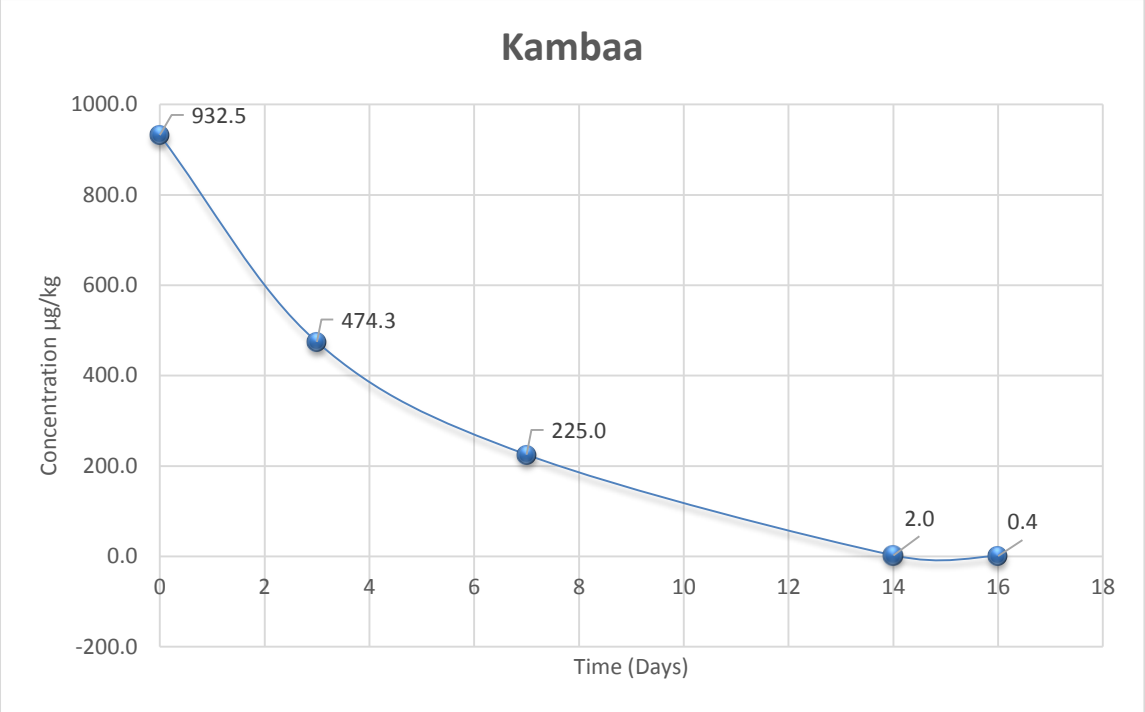

Figure 4.5B: Graph showing average reduction of carbendazim concentration against time for Kambaa Rodazim SC samples (Short and Long rain season).

Table 4.3: Mean for residue concentration at 0 to 16 days after application of pesticide.

Residue concentration (µg/kg) ±4 µg/kg									
	Kambaa				**Naivasha**				**Average**
Day/Sample	CS1	CS2	RS1	RS2	CS1	CS2	RS1	RS2	
0	1260.7	1039.4	645.9	825.7	970.3	678.4	675.2	905.5	**875.1**
3	496.2	492.8	349.0	455.8	467.3	368.2	341.0	576.3	**443.3**
7	220.1	244.1	180.2	205.8	188.5	134.5	210.9	192.8	**197.1**
14	5.7	3.9	1.5	0.0	31.6	15.7	0.0	0.0	**7.3**
16	5.7	0.8	0.0	0.0	0.7	0.6	0.0	0.0	**1.0**

Note: CS1; Chariot 500 SC Season 1, CS2; Chariot 500 Season 2; RS1; Rodazim SC Season 1, RS2, Rodazim SC Season 2.

The initial average deposition of carbendazim was 875.2 µg/kg at 0 day and 1.0 µg/kg at day 16 after application as show in table 4.3. The concentration of carbendazim decreased over time.

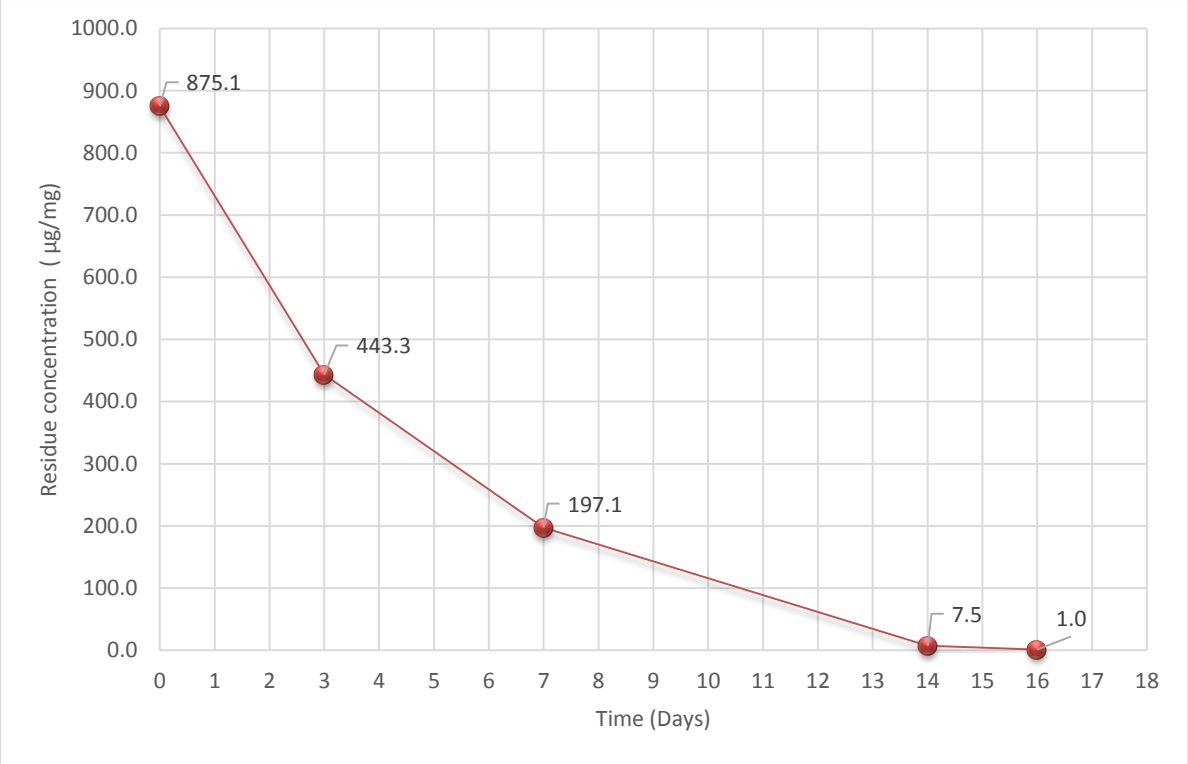

Figure 4.6: A graph of carbendazim concentration against time showing the trends of carbendazim dissipation**.**

The mean of the determined residue was plotted against time. Figure 4.6 shows that there was rapid dissipation of carbendazim for the first 3 days after application. This could be attributed to the half-life of the molecule in French beans

The data obtained in Tables 4.3 was fitted into Langmuir-Hinshelwood kinetic model for reaction rate dependence on initial reactant concentration (Karl *et al.*, 2013) to obtain rate constant (K_{obs}) and half-life ($t_{1/2}$).

$r = dC/dt = kKC/(1+ KC_o)$ ……Equation 4.1

Where r is the rate of reaction (mol/L.min^{-1}), C is the equilibrium concentration of reagent (ml/L), t is the time (min), k is the rate constant (l/min), and K is the Langmuir constant (L/mol). When the initial concentration C_o is ‹‹‹‹1, the denominator in equation (1) above was treated as 1, and the equation simplified to a first-order equation.

$dC/dt=kKC$……...Equation 4.2

$dC/C = kKdt$……...Equation 4.3

Integrating equation 3 and writing the rate law using exponents and logarithms gives

$C_t = C_o e^{-kt}$ ……Equation 4.4

$InC_t = InC_0 - K_{obs}X_t$ ……Equation 4.5

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

Where; C_t= pesticide concentration at time, t

K_{obs}= first order rate constant

t= time in Days

C_o= the original carbendazim concentration.

Consider the half-life of the reaction where the remaining concentration of the pesticide is half the original amount; $C_t = C_o/2$ and substituting in equation (1)

$\ln(C_o/2C_o) = Kt_{1/2}$……...Equation 4.6

$\ln 0.5 = -Kt_{1/2}$ …….. ..Equation 4.7

$-0.693/K = t_{1/2}$ ……...Equation 4.8

Based on first order kinetic, a plot of negative ln concentration of residues versus time t (days) K_{obs} is calculate from the graph (Figure 4.7)

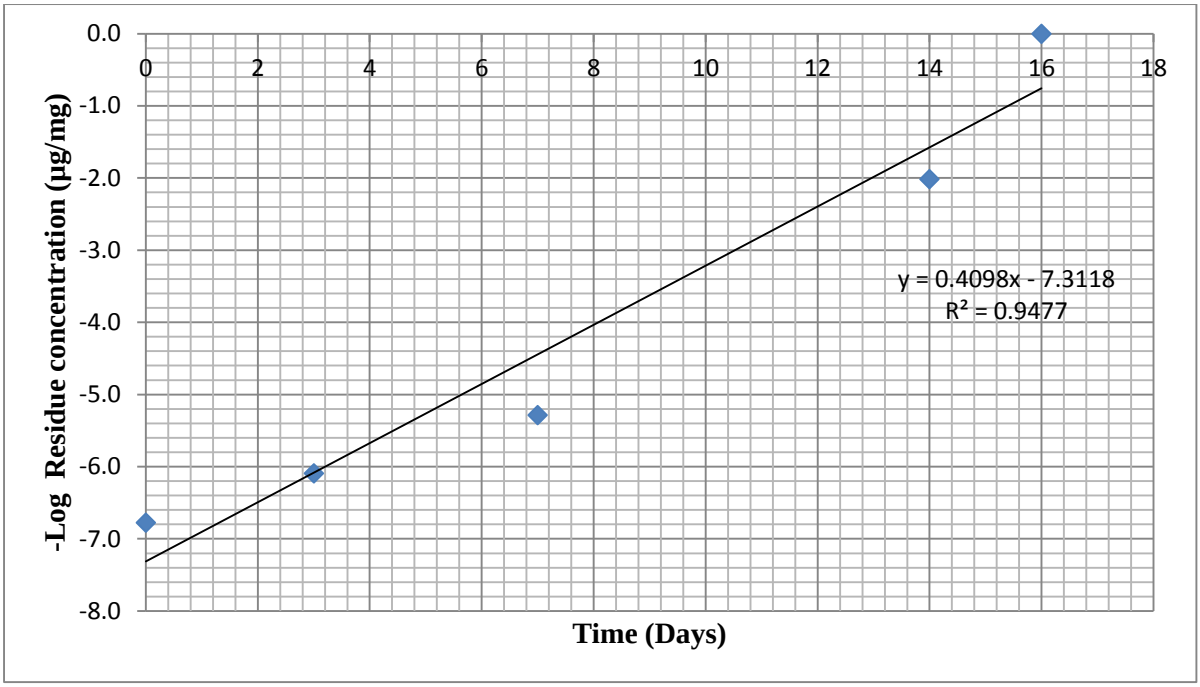

Figure 4.7: Regression curve for disappearance of carbendazim in French beans.

Figure 4.7 shows that the plot of In (Ct) verses time (t) of the carbendazim residue concentration determined gives a straight line with a R^2 0.947. A regression equation of y = 0.409x-7.311 with a gradient of 0.409 which is equivalent to the constant K_{obs} was obtained. In this study the degradation of carbendazim follows Langmuir-Hinshelwood kinetic equation and using equation (8) the half-life of carbendazim in French beans was found to be 1.7 days.

4.6 Pre-harvest interval for Carbendazim in French beans

Chariot 500SC and Rodazim SC formulation of carbendazim showed a similar pattern of degradation for season 1 and 2 in Kambaa and Naivasha sites. The EU set MRLS for French beans (beans with pods) according (EU) regulation 369/2005 is set at 200µg/kg. The pre harvest interval (PHI) of carbendazim was calculated at 50% of the EU set MRLs using equation (5) ($InC_t = InC_0 – K_{obs}X_t$). Where $C_t$is the calculated concentration at 50% of MRLs for French beans (100µg/kg), C_0 is the average deposition of carbendazim obtained at time zero in the study and $K_{obs\ is}$ the gradient obtained in Figure 4.7. In this study the PHI for carbendazim in French beans was found to be 5.4 day after application. This indicated that pesticide residue on or in French beans treated with carbendazim at a rate of 6.25 kg per hectare for the control of fungal diseases and following good agricultural practice (GAP) will be below the EU legally accepted MRLs of 200µg/kg 5 days after application.

4.7 National maximum residue levels tolerance for carbendazim in French beans.

Kenya lacks national maximum residue levels (MRLs) tolerance for carbendazim residue allowed in French beans which is an important tool for ensuring compliance with market requirements for market access. National maximum residue level tolerance acts as a buffer pesticide residue level to minimise the risk of Kenyan produce from being intercepted at the export market which can lead to a considerable economic losses if the maximum residue is exceeded.

The National tolerance was calculated at 1 day plus the determined PHI using equation (5) ($InC_t = InC_0 - K_{obs}X_t$), where X_t is 6 days, C_0 is the average deposition of carbendazim obtained at time zero in the study and $K_{obs\ is}$ the gradient obtained according to Figure 4.7. In this study the national pesticide tolerance for carbendazim in French beans was found to be 74µg/kg. This is expected to reduce the chances of having non complying produce with EU set MRLs entering the market. The level of compliances will increase and the risk of losing the EU market due to MRLs exceedances will be reduced. This will lead to spur sustainable job creation in the rural areas and spur economic growth in the country.

4.8 Statistical analysis of the data.

Analysis of Variance (ANOVA) statistical tool in Microsoft excel was used to determine if there was correlation between breakdowns of carbendazim in French beans with the type of formulation or region. Two-way ANOVA with replication was used was used to compare the breakdown of carbendazim based on pesticide formulation (Chariot 500 SC and Rodazim) and region (Kambaa and Naivasha). The aim was to determine if there is statistically significant influence of carbendazim breakdown by the two independent variables (Formulations and GEOGRAPHICAL region). The summary output from the ANOVA analysis in Table 13A, Appendix 13 showed that; the F calculated (Formulation) was $0.02 < 4.14$ F-critical and F calculate (region) $0.17 < 2.9$ (F-critical), therefore conclude with 95% confidence that there are no significance difference in carbendazim breakdown of carbendazim in French beans within the formulation and region.

The output also shows that the due to the interaction between the two variables (formulation and region) was F calculate $0.13 < 2.90$ F-critical an indication that there are no significance difference at 95% confidence. Therefore it is concluded that there is correlation between the breakdown of carbendazim in French beans with formulation and region.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The data collected through the questionnaire showed that exporters of French beans deals with more than one variety. The choice of variety is guided by factors such as; target market, client specification, and resistance to pest, diseases and drought. The discussion of the questionnaire data also highlighted that the exporters were aware of fungal diseases affecting French beans. To control the pest, exporters used pesticide such as carbendazim. Observing PHI is critical to avoid exceedance of the pesticide residue above the MRLs.

The current study revealed that, dissipation of carbendazim pesticide in chariot 500SC and Rodazim SC in French beans follow a similar pattern. The pattern was comparable in short and long rains seasons. There was significant degradation of more than 36.2% of carbendazim in the first 3 day after application and more 95% in the second week. Due to lack of harmonized pre-harvest interval (PHI), carbendazim has led to French beans border rejections and increased border controls imposed on Kenyan beans exports to EU. Half-life of 1.7 days for carbendazim in French bean was obtained from the study. Using the half-life, 5 days after application of carbendazim according to the right dosage and technique was found to be long enough to reduce the pesticide residue below the EU legally accepted MRLs of 200µg/kg in French beans. This period of time is referred to as PHI. It minimizes the probability of MRLs exceedances which reduces the level of human, animal and environment exposure to carbendazim and increases the level of compliance. Therefore 5 day PHI is considered as the most appropriate for carbendazim in French beans.

National maximum residue limits tolerance is a buffer level which is set below the MRLs. It will help the government to monitor the compliance and take action before an incident of noncompliance occurs. The country lacks field trial data on the dissipation of carbendazim in French beans; therefore there is high risk of Kenyan produce not meeting the market requirements due to international or regional regulations on pesticide residue. The residue detected after 14 days was way below the EU legally accepted maximum residue level in beans with pods (French beans). A national maximum residue tolerance is a buffer MRLs level which is set below the international or trading MRLs. Detection at this level triggers regulatory action to be taken to reduce the risk of MRLs exceedance and avoid non-

compliances. A 74µg/kg (0.074ppm) National maximum residue level tolerance determine at the PHI plus 1 day was found suitable.

5.2 Recommendations.

- Further studies should be conducted on other pesticides used on French beans and particular focus on: Chlorpyrifos, Carbofuran, Cypermethrin, Cyfluthrine, Teboconazole and Azoxystrobin.
- There is need to build capacity in the regulatory competent bodies involved in pesticide use, registration and monitoring in-order to establish national pesticide tolerance for all pesticides used as plant protection products in Kenya.
- Policy framework should be instituted focusing on research and promotion on use of Bio pesticides. Bio pesticides have strong specificity to target pests, safe to humans, animals and are pollution free. The more than 80% French beans' production for export that is done by small scale farmers in the rural areas would benefit from the regulations.
- There is need to educate farmers on safe use of pesticides and to observe PHI in order to protect local consumers and environment.
- There is need to educate farmers to use integrated pesticide management (IPM) in order to protect local consumers and environment.

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APPENDICES

Appendix 1: EU maximum residue limits setting.

Figure 1A: MRLs setting process followed by the EU.

Appendix 2: French beans varieties and pesticide formulation selection.

Table 2A: Questionnaire for French beans and pesticides companies.

Part A: EXPORTER INFORMATION		
1	Company Name	
2	Export license no:	
3	Physical Address:	
4	Contact person name: Email address:	Cell phone no.
Part B: PRODUCTION INFORMATION		
5	Are you company licensed to export beans with pods (Tick where appropriate). Yes ( ) or No ( )	
6	Who are your producers? (Tick one or more). a) Own farms ( ) b) Contracted famers ( ) c) brokers ( )	
7	What is the approximate volume your company exports per week? (Tick where appropriate) a) Less than 500kg ( ) b) 500-1000kg ( ) c) 1000kg to 2000kg ( ) d) More than 2000kg ( ) e) Others ( )	

8	What are the French beans varieties do you grow for export? Give in order of volumes from the highest. a)……. b)……. c)……. d)……. e)…….
	What are the fungi diseases experienced when growing beans with pods? a)……. b)……. c)……. d)……. e)…….
	What pesticide do you use to control the fungi diseases? Give the active and brand. a)……. b)……. c)……. d)…….

Table 2B: List of the interviewed companies and their responses.

	CONTACT PERSON	COMPANY	LICENSED	SOURCE OF PRODUCE	BATCHS EXPORTED(KG)	VARIETY	FUNGAL DISEASES	PESTICIDE USED
1	Mburu	Afya Fresh	x	Contracted farmers	≥2000	Army	Anthracnose	Score
						Samantha	Rust	Azoxystrobin
						Serengeti		Carbendazim
						Vanilla		
2	Meshack	Oka Fresh	x	Contracted farmers	1000-2000	Samantha	Rust	Difenoconazole
				Own farm		Army	Blight	Copper
						Star	Angular leaf spot	Carbendazim
3	Mohamed	Sacco Fresh	x	Own farm	≥2000	Army	Rust	Azoxystrobin
				Contracted farmers		Samantha	Hallow blight	Tebuconazole
						Vanilla	Powderly mildew	Sulfur
						Teresa	Angular leaf spot	Carbendazim
						Serengeti		
4	Muteti	Summer fresh	x	Contracted farmers	500-1000	Samantha	Bacteria wilt	sulfur
						Serengeti	Dumping off	Azoxystrobin
						Teresa	Powdery Mildew	Copper
						Boston	Angular leaf spot	Potassium diphosphate

						Star	Blight	
5	Waitakere	Mara Farm	x	Own farm	≥2000	Teresa	Rust	Azoxystrobin
				Contracted farmers		Serengeti	Powdery Mildew	Thiomethoxam
						Vanilla	Angular leaf spot	Carbendazim
6	Gideon	Intervege	x	Contracted farmers	≥2000	Serengeti	Anthracnose	Difenoconazole
						Samantha	Dumping off	Tebuconazole
						Army	Blight	Azoxystrobin
						Vanilla	Rust	
						Star	Fusarium wilt	
7	Samuel	Farmer	x	Own farm	500-1000	Serengeti	Rust	Azoxystrobin
						Vanilla	Anthracnose	Carbendazim
						Star	Blight	Difeconazole
						Army	Dumping off	Copper
							Fusarium wilt	
8	Mbivya	Vert	x	Contracted farmers	1000-2000	Serengeti	Rust	Azoxystrobin
						Vanilla	Angular leaf spot	Copper
						Oreandi	Anthracnose	Carbendazim
						Star		
						Goal		
9	Ondogo	Meru green	x	Contracted farmers	≥2000	Teresa	Blight	Azoxystrobin
				Own farmers		Army	Anthracnose	Carbendazim
						Samantha		Copper

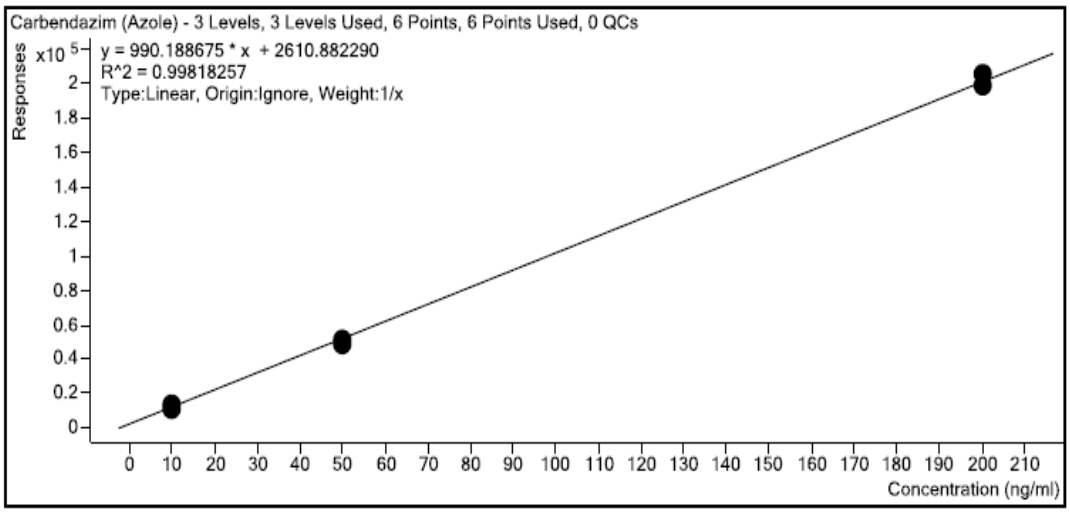
10	Poline	Sian	x	Contracted farmers	≥2000	Vanilla	Blight	Azoxystrobin
				Marketing agent		Teresa	Angular leaf spot	Carbendazim
						Moonstone	Rust	Copper
						Star	Anthracnose	
						Serengeti		
11	Habel	Vegpro	x	Contracted farmers	≥2000	Teresa	Powdery mildew	Azoxystrobin
				Own farm		Serengeti	Downy mildew	Difenoconazole
						Vanilla	Ascochytae	Metalaxyl_Mancozeb
							Rust	Tebuconazole
							Angular leaf spot	
12	Japheth	Keitt	x	Contracted farmers	≥2000	Star	Rust	Azoxystrobin
						Serengeti	Anthracnose	Difenoconazole
						Samantha	Hallow blight	Tebuconazole
13		Flamingo	x	Contracted farmers	≥2000	Samantha	Rust	Azoxystrobin
				Own farm		Serengeti	Angular leaf spot	Carbendazim
						Vanilla	Anthracnose	Tebuconazole
14	Mwikali	Athi farm	x	Contracted farmers	≥2000	Star	Angular leaf spot	Carbendazim
				Own farm		Samantha	Rust	Metalaxyl_Mancozeb
						Serengeti	Downy mildew	
15	Kioko	Giraffe	x	Contracted farmers	≥2000	Serengeti	Anthracnose	Azoxystrobin
				Own farm		Vanilla	Blight	Thiomethoxam
						Teresa		

16	Antony	AAA	x	Own farm	≥2000	Vanilla	Rust	Azoxystrobin
						Army	Ascochytae	Carbendazim
							Powdery mildew	Difenoconazole
17	Ampfly	Kandia	x	Contracted farmers	1000-2000	Serengeti	Rust	Carbendazim
						Moonstone		Azoxystrobin
								Thiomethoxam
18	Joshua	EAGA	x	Contracted farmers	1000-2000	Samantha	Blight	Carbendazim
				Own farm		Serengeti	Downy mildew	Metalaxyl_Mancozeb
						Goal		
						Army		
19	David	Shalma	x	Contracted farmers	≥2000	Star	Angular Leaf spot	Azoxystrobin
				Own farm		Army	Anthracnose	Difenoconazole
						Vanilla	Rust	Tebuconazole
						Teresa		Carbendazim
20	Isaac	Sunrip	x	Contracted farmers	≥2000	Serengeti	Rust	Azoxystrobin
				Own farm		Vanilla	Powdery mildew	Carbendazim
						Army		
						Samantha		

Appendix 3: Standard calibration curve.

Batch Data Path		D:\MassHunter\Data\2016 samples\April\2004201601\Quant Results\2004201601.batch.bin	
Analysis Time	4-21-2016 10:38 AM	**Analyst Name**	admin
Report Time	4-21-2016 10:42 AM	**Reporter Name**	admin
Last Calib Update	4-21-2016 10:38 AM	**Batch State**	Processed
Quant Batch Version	B.05.02	**Quant Report Version**	B.05.02

Target Compound Carbendazim (Azole)



Calibration STD	Cal Type	Level	Enabled	Response	RF	RSD	Exp Conc
D:\MassHunter\Data\2016 samples\april\2004201601\2.d	Calibration	L1	☑	13889	1388.8986	11.19	10.0000
D:\MassHunter\Data\2016 samples\april\2004201601\37.d	Calibration	L1	☑	11851	1185.1421	11.19	10.0000
D:\MassHunter\Data\2016 samples\april\2004201601\3.d	Calibration	L2	☑	51216	1024.3196	3.86	50.0000
D:\MassHunter\Data\2016 samples\april\2004201601\38.d	Calibration	L2	☑	48497	969.9430	3.86	50.0000
D:\MassHunter\Data\2016 samples\april\2004201601\4.d	Calibration	L3	☑	199050	995.2496	2.45	200.0000
D:\MassHunter\Data\2016 samples\april\2004201601\39	Calibration	L3	☑	206060	1030.2998	2.45	200.0000

Figure 3A: Calibration curve for carbendazim standards used in determination of analyte concentration in the sample.

Appendix 4: Internal standard chromatogram

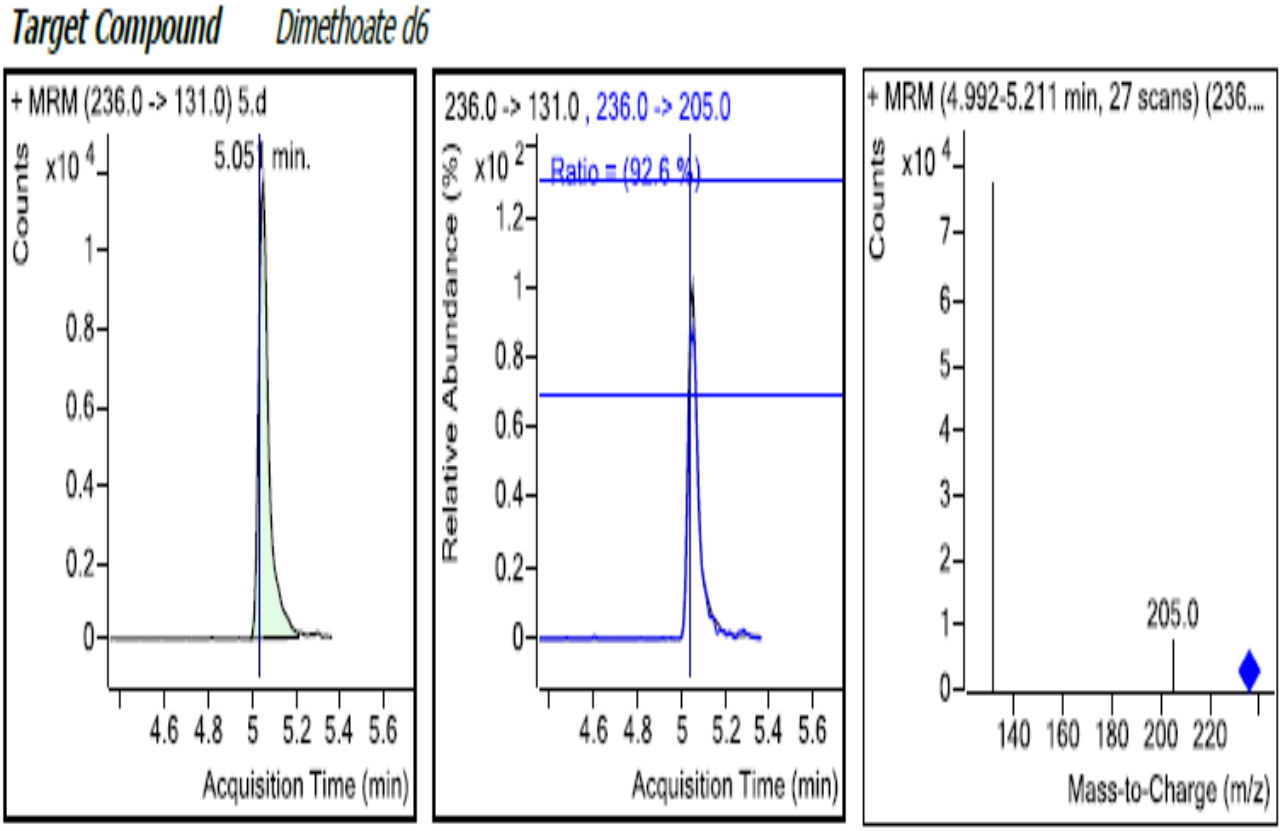
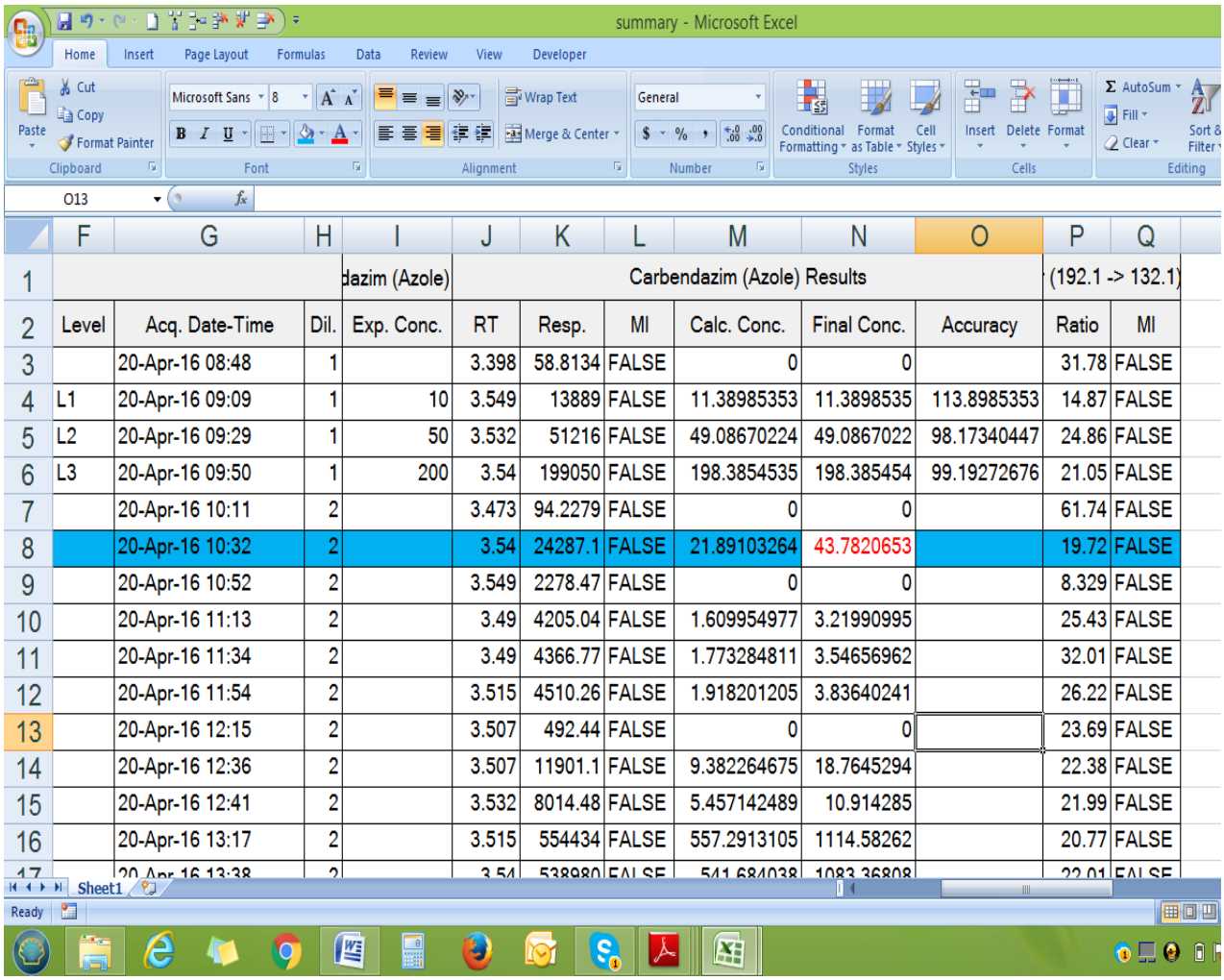


Figure 4 A: Chromatograms of Dimethoate D6 internal standard showing the transitions ions abundance, retention time and a total scan.

Appendix 5: Recovery data for spiked sample.

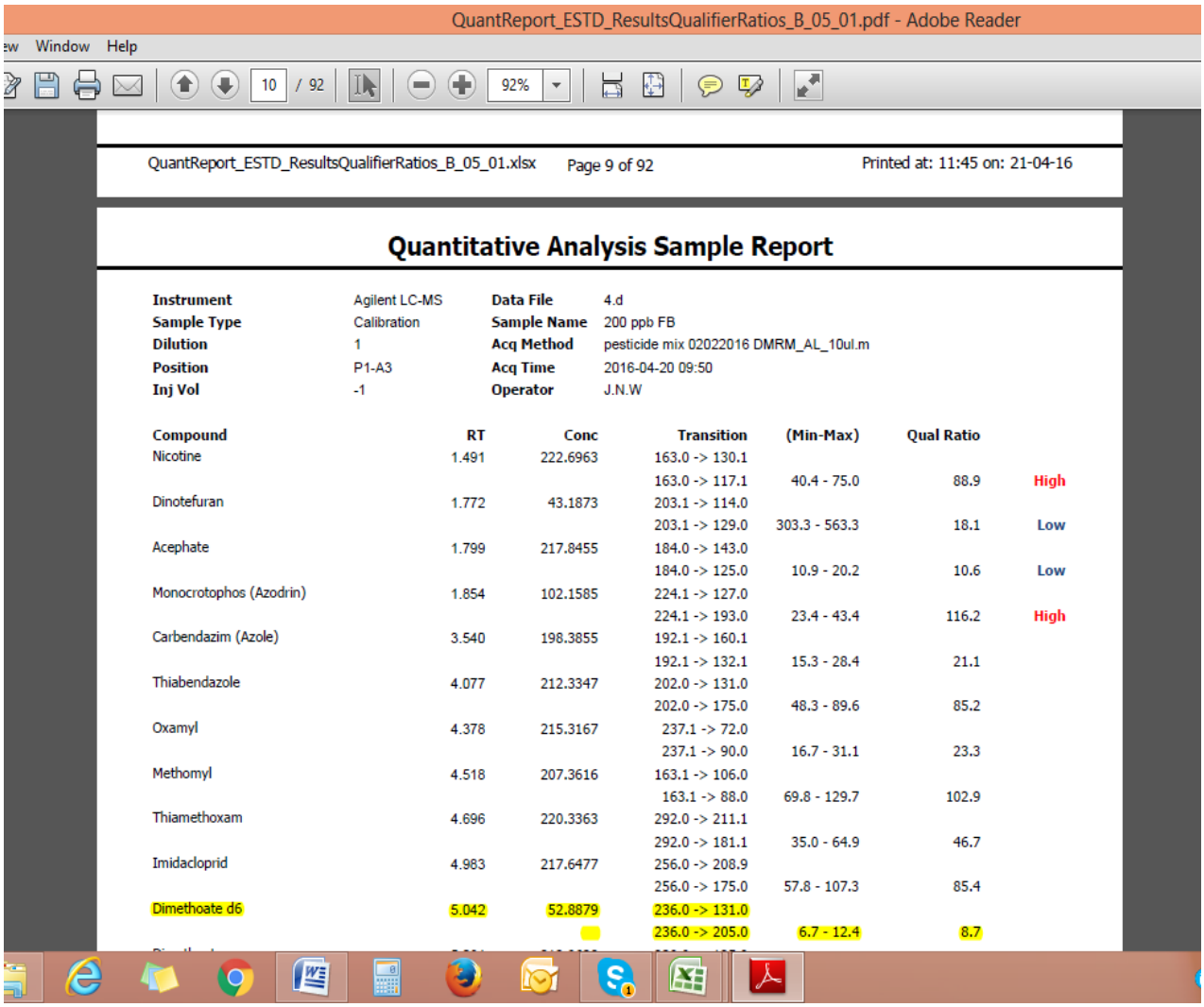


summary - Microsoft Excel

	F	G	H	I	J	K	L	M	N	O	P	Q
1				dazim (Azole)	Carbendazim (Azole) Results						(192.1 -> 132.1)	
2	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI
3		20-Apr-16 08:48	1		3.398	58.8134	FALSE	0	0		31.78	FALSE
4	L1	20-Apr-16 09:09	1	10	3.549	13889	FALSE	11.38985353	11.3898535	113.8985353	14.87	FALSE
5	L2	20-Apr-16 09:29	1	50	3.532	51216	FALSE	49.08670224	49.0867022	98.17340447	24.86	FALSE
6	L3	20-Apr-16 09:50	1	200	3.54	199050	FALSE	198.3854535	198.385454	99.19272676	21.05	FALSE
7		20-Apr-16 10:11	2		3.473	94.2279	FALSE	0	0		61.74	FALSE
8		20-Apr-16 10:32	2		3.54	24287.1	FALSE	21.89103264	43.7820653		19.72	FALSE
9		20-Apr-16 10:52	2		3.549	2278.47	FALSE	0	0		8.329	FALSE
10		20-Apr-16 11:13	2		3.49	4205.04	FALSE	1.609954977	3.21990995		25.43	FALSE
11		20-Apr-16 11:34	2		3.49	4366.77	FALSE	1.773284811	3.54656962		32.01	FALSE
12		20-Apr-16 11:54	2		3.515	4510.26	FALSE	1.918201205	3.83640241		26.22	FALSE
13		20-Apr-16 12:15	2		3.507	492.44	FALSE	0	0		23.69	FALSE
14		20-Apr-16 12:36	2		3.507	11901.1	FALSE	9.382264675	18.7645294		22.38	FALSE
15		20-Apr-16 12:41	2		3.532	8014.48	FALSE	5.457142489	10.914285		21.99	FALSE
16		20-Apr-16 13:17	2		3.515	554434	FALSE	557.2913105	1114.58262		20.77	FALSE
17		20-Apr-16 13:38	2		3.54	538980	FALSE	541.684038	1083.36808		22.01	FALSE

Figure 5A: Recovery data for carbendazim spiked at 50 µg/kg. The concentration of the recovery is shown in red.

Appendix 6: Recovery data for internal standard.



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QuantReport_ESTD_ResultsQualifierRatios_B_05_01.xlsx Page 9 of 92 Printed at: 11:45 on: 21-04-16

Quantitative Analysis Sample Report

Instrument	Agilent LC-MS	**Data File**	4.d
Sample Type	Calibration	**Sample Name**	200 ppb FB
Dilution	1	**Acq Method**	pesticide mix 02022016 DMRM_AL_10ul.m
Position	P1-A3	**Acq Time**	2016-04-20 09:50
Inj Vol	-1	**Operator**	J.N.W

Compound	RT	Conc	Transition	(Min-Max)	Qual Ratio	
Nicotine	1.491	222.6963	163.0 -> 130.1			
			163.0 -> 117.1	40.4 - 75.0	88.9	High
Dinotefuran	1.772	43.1873	203.1 -> 114.0			
			203.1 -> 129.0	303.3 - 563.3	18.1	Low
Acephate	1.799	217.8455	184.0 -> 143.0			
			184.0 -> 125.0	10.9 - 20.2	10.6	Low
Monocrotophos (Azodrin)	1.854	102.1585	224.1 -> 127.0			
			224.1 -> 193.0	23.4 - 43.4	116.2	High
Carbendazim (Azole)	3.540	198.3855	192.1 -> 160.1			
			192.1 -> 132.1	15.3 - 28.4	21.1	
Thiabendazole	4.077	212.3347	202.0 -> 131.0			
			202.0 -> 175.0	48.3 - 89.6	85.2	
Oxamyl	4.378	215.3167	237.1 -> 72.0			
			237.1 -> 90.0	16.7 - 31.1	23.3	
Methomyl	4.518	207.3616	163.1 -> 106.0			
			163.1 -> 88.0	69.8 - 129.7	102.9	
Thiamethoxam	4.696	220.3363	292.0 -> 211.1			
			292.0 -> 181.1	35.0 - 64.9	46.7	
Imidacloprid	4.983	217.6477	256.0 -> 208.9			
			256.0 -> 175.0	57.8 - 107.3	85.4	
Dimethoate d6	5.042	52.8879	236.0 -> 131.0			
			236.0 -> 205.0	6.7 - 12.4	8.7	

Figure 6A: Recovery data for Dimethoate D6. The yellow color shows the *M/Z* abundance ratio for the transitions.

Appendix 7: Outlier test

Table 7A: Testing for outlier in the determined concentration (µg/kg) by Grubbs test formula.

		0K1	0N1	3K1	3N1	7K1	7N1	14K1	14N1	16K1	16N1
Control	**C1**	1.07	1.00	0.98	0.32	0.50	0.25	0.77	0.58	0.50	0.53
	Av	1.04		0.65		0.38		0.67		0.51	
	S1	1114.58	1083.37	456.23	410.85	230.25	191.74	18.21	47.52	1.05	0.69
	S2	1406.89	857.22	526.45	565.30	211.14	192.27	25.68	28.79	0.76	0.52
Chariot 500SC	**S3**			505.91	425.70	219.02	181.36	9.11	18.35	15.37	0.86
	Ave	1260.74	970.29	496.20	467.28	220.14	188.46	17.67	31.55	5.73	0.69
	MIN	1114.58	857.22	456.23	410.85	211.14	181.36	9.11	18.35	0.76	0.52
	MAX	1406.89	1083.37	526.45	565.30	230.25	192.27	25.68	47.52	15.37	0.86
	SD	206.70	159.91	36.10	85.21	9.60	6.15	8.30	14.78	8.35	0.17
G critical	**CV**	0.16	0.16	0.07	0.18	0.04	0.03	0.47	0.47	1.46	0.25
1.15	**Outlier Min**			1.11	0.66	0.94	1.15	1.03	0.89	0.59	0.99
1.15	**Outlier Max**			0.84	1.15	1.05	0.62	0.97	1.08	1.15	1.01
	Outlier max										
	B1	1063.77	718.66	506.16	369.99	214.73	124.84	3.80	0.60	0.81	0.76
	B2	1014.98	638.11	551.69	375.79	191.74	146.91	4.14	32.56	1.38	0.27
Rodazim 500SC	**B3**			420.50	358.71	325.90	131.89	3.83	13.83	0.22	0.67
	Ave	1039.37	678.39	492.78	368.16	244.13	134.55	3.92	15.67	0.80	0.57
	MIN	1014.98	638.11	420.50	358.71	191.74	124.84	3.80	0.60	0.22	0.27
	MAX	1063.77	718.66	551.69	375.79	325.90	146.91	4.14	32.56	1.38	0.76
	SD	34.49	56.96	66.61	8.68	71.75	11.27	0.19	16.06	0.58	0.26
G critical	**CV**	0.03	0.08	0.14	0.02	0.29	0.08	0.05	1.02	0.72	0.46
1.15	**Outlier Min**			1.09	1.09	0.73	0.86	0.66	0.94	1.01	1.13

1.15	**Outlier Max**			0.88	0.88	1.14	1.10	1.15	1.05	0.99	0.75
		0K1	**0N1**	**3K1**	**3N1**	**7K1**	**7N1**	**14K1**	**14N1**	**16K1**	**16N1**
Control	**C**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Av	**0.00**		**0.00**		**0.00**		**0.00**			
	S1	623.19	638.08	373.93	379.62	200.11	198.57	0.25	0.00	0.00	0.00
	S2	668.56	712.32	337.93	345.74	212.65	200.75	3.76	0.00	0.00	0.00
Chariot 500SC	**S3**			335.22	340.97	127.87	233.32	0.43	0.00	0.00	0.00
	Ave	645.87	675.20	349.03	355.45	180.21	210.88	1.48	0.00	0.00	0.00
	MIN	623.19	638.08	335.22	340.97	200.11	198.57	0.25	0.00	0.00	0.00
	MAX	668.56	712.32	373.93	379.62	212.65	233.32	3.76	0.00	0.00	0.00
	SD	32.09	52.49	21.61	21.07	45.76	19.46	1.98	0.00	0.00	0.00
G critical	**CV**	0.05	0.08	0.06	0.06	0.25	0.09	1.33	#DIV/0!	#DIV/0!	#DIV/0!
1.15	**Outlier Min**			0.64	0.69	-0.43	0.63	0.62	#DIV/0!	#DIV/0!	#DIV/0!
1.15	**Outlier Max**			1.15	1.15	0.71	1.15	1.15	#DIV/0!	#DIV/0!	#DIV/0!
	Outlier max										
	B1	774.46	855.41	483.20	590.88	267.27	182.16	0.00	0.00	0.00	0.00
	B2	876.92	955.49	423.87	545.60	127.05	129.27	0.00	0.00	0.00	0.00
Rodazim 500SC	**B3**			460.39	592.32	223.08	266.96	0.00	0.00	0.00	0.00
	Ave	825.69	905.45	455.82	576.27	205.80	192.80	0.00	0.00	0.00	0.00
	MIN	774.46	855.41	423.87	545.60	127.05	129.27	0.00	0.00	0.00	0.00
	MAX	876.92	955.49	483.20	592.32	267.27	266.96	0.00	0.00	0.00	0.00
	SD	72.45	70.77	29.92	26.57	71.69	69.46	0.00	0.00	0.00	0.00
G critical	**CV**	0.09	0.08	0.07	0.05	0.35	0.36	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
1.15	**Outlier Min**			1.07	1.15	1.10	0.91	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
1.15	**Outlier Max**			0.91	0.60	0.86	1.07	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	Outlier max										

Appendix 8: Samples tested

Table 8A: Summary of the concentration of carbendazim determined in samples tested in the study.

	Sample code	Carbendazim brand	Season	Concentration (µg/kg)
1	OK1C1	NA	Season 1	1.07
2	ON1C1	NA	Season 1	1.00
3	OK1S1	Chariot 50 SC	Season 1	1114.58
4	OK1S2	Chariot 50 SC	Season 1	1406.89
5	OK1B2	Rodazim 50 SC	Season 1	1063.77
6	OK1B3	Rodazim 50 SC	Season 1	1014.98
7	ON1S1	Chariot 50 SC	Season 1	1083.37
8	ON1S2	Chariot 50 SC	Season 1	857.22
9	ON1B2	Rodazim 50 SC	Season 1	718.66
10	ON1B3	Rodazim 50 SC	Season 1	638.11
11	3K1C1	NA	Season 1	0.98
12	3N1C2	NA	Season 1	0.32
13	3K1S1	Chariot 50 SC	Season 1	456.23
14	3K1S2	Chariot 50 SC	Season 1	526.45
15	3K1S3	Chariot 50 SC	Season 1	505.91
16	3K1B1	Rodazim 50 SC	Season 1	506.16
17	3K1B2	Rodazim 50 SC	Season 1	551.69
18	3K1B3	Rodazim 50 SC	Season 1	420.50
19	3N1S1	Chariot 50 SC	Season 1	410.85
20	3N1S2	Chariot 50 SC	Season 1	565.30
21	3N1S3	Chariot 50 SC	Season 1	425.70
22	3N1B1	Rodazim 50 SC	Season 1	369.99
23	3N1B2	Rodazim 50 SC	Season 1	375.79
24	3N1B3	Rodazim 50 SC	Season 1	358.71
25	7K1C1	NA	Season 1	0.50
26	7N1C2	NA	Season 1	0.25
27	7K1S1	Chariot 50 SC	Season 1	230.23

28	7K1S2	Chariot 50 SC	Season 1	211.14
29	7K1S3	Chariot 50 SC	Season 1	219.02
30	7K1B1	Rodazim 50 SC	Season 1	214.73
31	7K1B2	Rodazim 50 SC	Season 1	191.74
32	7K1B3	Rodazim 50 SC	Season 1	325.90
33	7N1S1	Chariot 50 SC	Season 1	191.74
34	7N1S2	Chariot 50 SC	Season 1	192.27
35	7N1S3	Chariot 50 SC	Season 1	181.36
36	7N1B1	Rodazim 50 SC	Season 1	124.84
37	7N1B2	Rodazim 50 SC	Season 1	146.91
38	7N1B3	Rodazim 50 SC	Season 1	131.89
39	14K1C1	NA	Season 1	0.77
40	14N1C2	NA	Season 1	0.58
41	14K1S1	Chariot 50 SC	Season 1	18.21
42	14K1S2	Chariot 50 SC	Season 1	25.68
43	14K1S3	Chariot 50 SC	Season 1	9.11
44	14K1B1	Rodazim 50 SC	Season 1	3.80
45	14K1B2	Rodazim 50 SC	Season 1	4.14
46	14K1B3	Rodazim 50 SC	Season 1	3.83
47	14N1S1	Chariot 50 SC	Season 1	47.52
48	14N1S2	Chariot 50 SC	Season 1	28.79
49	14N1S3	Chariot 50 SC	Season 1	18.35
50	14N1B1	Rodazim 50 SC	Season 1	0.60
51	14N1B2	Rodazim 50 SC	Season 1	32.56
52	14N1B3	Rodazim 50 SC	Season 1	13.83
53	16K1C1	NA	Season 1	0.50
54	16K1C2	NA	Season 1	0.53
55	16K1S1	Chariot 50 SC	Season 1	1.05
56	16K1S2	Chariot 50 SC	Season 1	0.76
57	16K1S3	Chariot 50 SC	Season 1	15.37
58	16K1B1	Rodazim 50 SC	Season 1	0.81
59	16K1B2	Rodazim 50 SC	Season 1	1.38

60	16K1B3	Rodazim 50 SC	Season 1	0.22
61	16N1S1	Chariot 50 SC	Season 1	0.69
62	16N1S2	Chariot 50 SC	Season 1	0.52
63	16N1S3	Chariot 50 SC	Season 1	0.86
64	16N1B1	Rodazim 50 SC	Season 1	0.76
65	6N1B2	Rodazim 50 SC	Season 1	0.27
66	16N1B3	Rodazim 50 SC	Season 1	0.67
67	OK2C1	NA	Season 2	ND
68	OK2C2	NA	Season 2	ND
69	OK2S1	Chariot 50 SC	Season 2	623.19
70	OK2S2	Chariot 50 SC	Season 2	668.56
71	OK2B1	Rodazim 50 SC	Season 2	774.46
72	OK2B2	Rodazim 50 SC	Season 2	876.92
73	ON2S1	Chariot 50 SC	Season 2	638.08
74	ON2S2	Chariot 50 SC	Season 2	712.32
75	ON2B1	Rodazim 50 SC	Season 2	855.41
76	ON2B2	Rodazim 50 SC	Season 2	955.49
77	3K2C1	NA	Season 2	ND
78	3K2C2	NA	Season 2	ND
79	3K2S1	Chariot 50 SC	Season 2	373.93
80	3K2S2	Chariot 50 SC	Season 2	337.93
81	3K2S3	Chariot 50 SC	Season 2	335.22
82	3K2B1	Rodazim 50 SC	Season 2	483.20
83	3K2B2	Rodazim 50 SC	Season 2	423.87
84	3K2B3	Rodazim 50 SC	Season 2	460.39
85	3N2S1	Chariot 50 SC	Season 2	379.62
86	3N2S2	Chariot 50 SC	Season 2	345.74
87	3N2S3	Chariot 50 SC	Season 2	340.97
88	3N2B1	Rodazim 50 SC	Season 2	590.88
89	3N2B2	Rodazim 50 SC	Season 2	545.60
90	3N2B3	Rodazim 50 SC	Season 2	592.32
91	7K2C1	NA	Season 2	ND

92	7K2C2	NA	Season 2	ND
93	7K2S1	Chariot 50 SC	Season 2	200.11
94	7K2S2	Chariot 50 SC	Season 2	212.65
95	7K2S3	Chariot 50 SC	Season 2	127.87
96	7K2B1	Rodazim 50 SC	Season 2	267.27
97	7K2B2	Rodazim 50 SC	Season 2	127.05
98	7K2B3	Rodazim 50 SC	Season 2	223.08
99	7N2S1	Chariot 50 SC	Season 2	198.57
100	7N2S2	Chariot 50 SC	Season 2	200.75
101	7N2S3	Chariot 50 SC	Season 2	233.32
102	7N2B1	Rodazim 50 SC	Season 2	182.16
103	7N2B2	Rodazim 50 SC	Season 2	129.27
104	7N2B3	Rodazim 50 SC	Season 2	266.96
105	14K2C1	NA	Season 2	ND
106	14K2C2	NA	Season 2	ND
107	14K2S1	Chariot 50 SC	Season 2	ND
108	14K2S2	Chariot 50 SC	Season 2	ND
109	14K2S3	Chariot 50 SC	Season 2	0.25
110	14K2B1	Rodazim 50 SC	Season 2	3.76
111	14K2B2	Rodazim 50 SC	Season 2	0.46
112	14K2B3	Rodazim 50 SC	Season 2	ND
113	14N2S1	Chariot 50 SC	Season 2	ND
114	14N2S2	Chariot 50 SC	Season 2	ND
115	14N2S3	Chariot 50 SC	Season 2	ND
116	14N2B1	Rodazim 50 SC	Season 2	ND
117	14N2B2	Rodazim 50 SC	Season 2	ND
118	14N2B3	Rodazim 50 SC	Season 2	ND
119	16K2C1	NA	Season 2	ND
120	16K2C2	NA	Season 2	ND
121	16K2S1	Chariot 50 SC	Season 1	ND
121	16K2S2	Chariot 50 SC	Season 1	ND
123	16K2S3	Chariot 50 SC	Season 1	ND

124	16K2B1	Rodazim 50 SC	Season 1	ND
125	16K2B2	Rodazim 50 SC	Season 1	ND
226	16K2B3	Rodazim 50 SC	Season 1	ND
127	16N2S1	Chariot 50 SC	Season 1	ND
128	16N2S2	Chariot 50 SC	Season 1	ND
129	16N2S3	Chariot 50 SC	Season 1	ND
130	16N2B1	Rodazim 50 SC	Season 1	ND
131	6N2B2	Rodazim 50 SC	Season 1	ND
132	16N2B3	Rodazim 50 SC	Season 1	ND

Appendix 9: Sample total scan chromatogram.

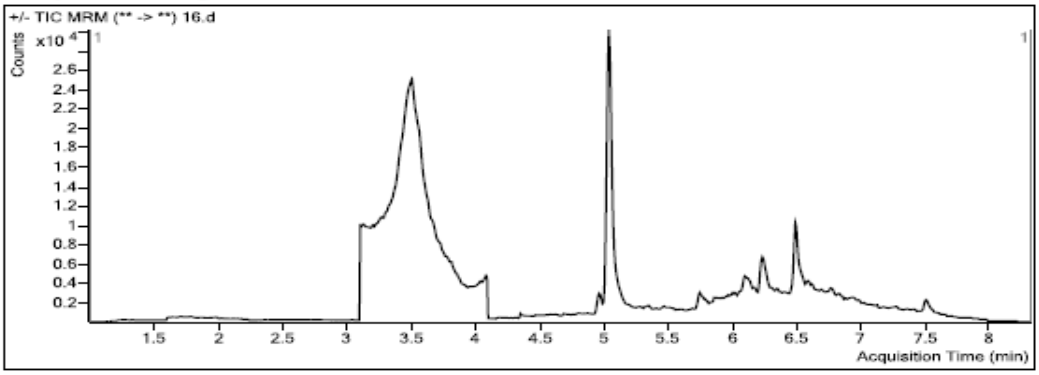
Quantitative Analysis Sample Report

Batch Data Path	D:\MassHunter\Data\2016 samples\april\0104201601_040416.s\QuantResults\0104201601.batch.bin		
Analysis Time	4-8-2016 8:08 AM	**Analyst Name**	admin
Report Time	8-24-2016 4:27 PM	**Reporter Name**	admin
Last Calib Update	4-8-2016 8:08 AM	**Batch State**	Processed

Analysis Info

Acq Time	2016-04-06 17:49	**Data File**	16.d
Position	P1-B6	**Sample Name**	sample67
Dilution	2	**Sample Info**	
Inj Vol	-1.00	**Acq Method File**	pesticide mix 02022016 DMRM_AL_10ul.m
Sample Type	Sample	**Comment**	new internal standard dimethoate d6

Sample Chromatogram



Quantitation Results

Compound	RT	Response	Conc	Accuracy
Nicotine	1.659	19	6.1561	
Acephate	1.716	339	0.0000	
Dinotefuran	1.780	183	0.0000	
Monocrotophos (Azodrin)	1.879	9	1231.4727	
Carbendazim (Azole)	3.507	214636	191.7441	
Thiabendazole	3.960	23	0.0000	

Figure 9A: Chromatogram showing the retention of carbendazim at 3.5 min marked in blue and determined concentration.

Appendix 10: Sample and spiked sample chromatogram

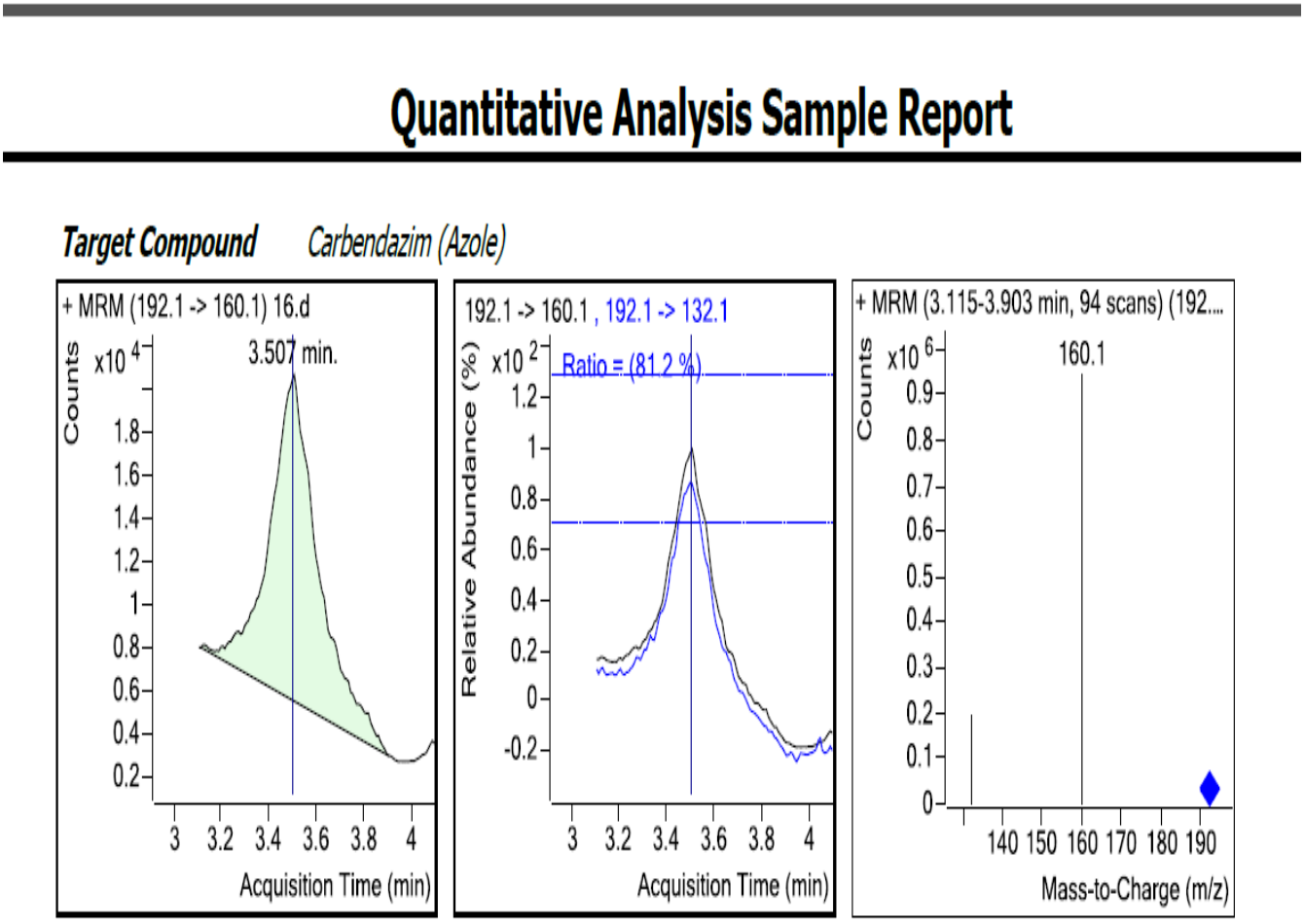


Figure 10A: Chromatograms of a sample showing carbendazim transitions ions abundance, retention time and a total scan.

Quantitative Analysis Sample Report

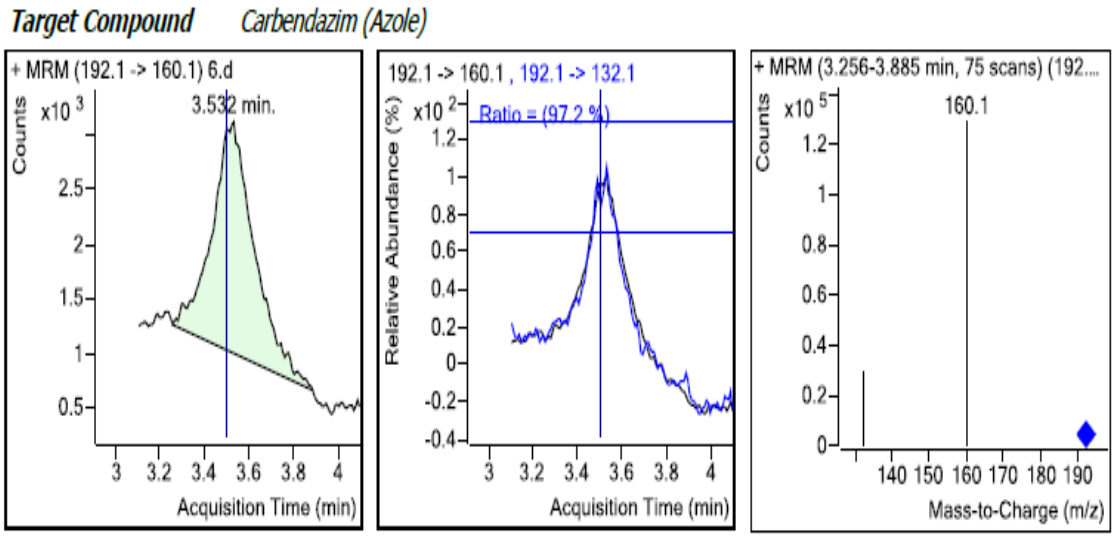


Figure 10B: Chromatograms of a spiked sample showing carbendazim transitions ions abundance, retention time and a total scan.

Appendix 11: ANNOVA test for variance

Table 11A: Summary of determined concentration of carbendazim at different season and sites

	Kambaa		Naivasha	
Pesticide	Season 1	Season 2	Season 1	Season 2
Chariot 500 SC	1260.7	645.9	970.3	675.2
	496.2	349	467.3	341
	220.1	180.2	188.5	210.9
	17.7	1.5	31.6	0
	5.7	0	0.7	0
Rodazim SC	1039.4	855.7	678.4	905.5
	492.8	455.8	368.2	576.3
	244.1	205.8	134.5	192.8
	3.9	0	15.7	0
	0.8	0	0.6	0

Table 11B: Summary of the two ANNOVA showing calculated variance

Anova: Two-Factor With Replication

	SUMMARY	Season 1	Season 2	Season 1	Season 2	Total	
1	*Chariot 500 SC*						
	Count	5	5	5	5	20	
	Sum	2000.4	1176.6	1658.4	1227.1	6062.5	
	Average	400.08	235.32	331.68	245.42	303.125	
	Variance	271012.172	73646.147	161581.142	78874.992	127956.4757	
2	*Rodazim SC*						
	Count	5	5	5	5	20	
	Sum	1781	1517.3	1197.4	1674.6	6170.3	
	Average	356.2	303.46	239.48	334.92	308.515	
	Variance	187103.165	130472.478	81845.387	157091.687	119203.3266	
	Total						
	Count	10	10	10	10		
	Sum	3781.4	2693.9	2855.8	2901.7		
	Average	378.14	269.39	285.58	290.17		
	Variance	204141.6649	92009.12767	110550.9129	107099.149		

	ANOVA						
	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	Sample	290.521	1	290.521	0.002035838	**0.964291849**	4.149097409
	Columns	72119.074	3	24039.69133	0.168459139	**0.916876123**	2.901119588
	Interaction	57408.489	3	19136.163	0.13409746	**0.93901174**	2.901119588
	Within	4566508.68	32	142703.3963			
	Total	4696326.764	39				