

**RESIDUAL DIAZINON IN *Brassica oleracea* var. *Acephala* AND INFLUENCE OF
APPLICATION CONDITIONS ON ITS LEVELS IN KIMIRA-OLUCH
SMALLHOLDER FARMERS IMPROVEMENT PROJECT, KENYA**

BY

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DECLARATION

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This thesis is my original work and has not been presented for the award of a degree in any other University.

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DEDICATION

This study is dedicated to my beloved family (Edith, Beverly, Daniel and David) and the West Kenya Union Conference of Seventh-day Adventists, who supported my studies.

ABSTRACT

Use of pesticides in agriculture often causes residual effects on crops with potential health risks. Diazinon, a synthetic pesticide whose dietary exposures have been associated with human health impacts is popular in the production of *Brassica oleracea var. acephala* at Kimira-Oluch Smallholder Farmers Improvement Project (KOSFIP), Kenya. Diazinon has relatively long pre-harvest interval (PHI) which farmers may not be observing. Levels of diazinon residues and associated health risks in farm-gate *Brassica oleracea var. acephala* are unknown. There is no available data on how application conditions influence the residue levels. This study quantified the residual diazinon levels in farm-gate baskets of the vegetable; influence of rate of application on residual levels at PHI; and influence of post-application duration on residual levels at different application rates. Cross-sectional survey based on purposive snowball sampling identified 40 of 45 farms from which samples were collected in triplicate. Randomized complete block design arranged in 5x8 split-plot controlled experiments were used to determine the influence of diazinon application rates and PHI conditions on residual levels. Main treatments were 0.0000, 0.6125, 1.2500, 1.8625 and 2.5000 litres ha⁻¹, respectively, split for 0, 5, 10, 13, 16, 18, 20 and 21 days post application harvesting. Samples were prepared using QuEChERS method, and subjected to LC-ESI-MS/MS analysis. Farm-gate samples had residual diazinon levels (92.5%) of which 67.57% were above the Codex Maximum Residue Limits. Of the samples, 40% had Health Risk Index >1.0, higher than most previous studies. Malpractices against Good Agricultural Practices (GAPs) may be responsible for higher residual levels with consequent increased health risks to consumers. The results demonstrate the need for surveillance and increased farmer education to reduce diazinon residue levels in the farm-gate vegetables. Residual diazinon levels increased ($P \leq 0.05$) with increasing rates of application and were inversely proportional to increasing PHI in agreement with previous studies. Application rates ≥ 1.25 litres/ha required longer PHI than the labeled, suggesting that the recommended PHI is inappropriate for the vegetable in the study area. A review of PHI from 12 to 14 is recommended for rate of 1.25 litres/ha. The use of diazinon on *Brassica oleracea var. acephala* at KOSFIP should be discouraged and alternative pesticides be used according to GAPs.

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ABBREVIATIONS AND ACRONYMS

Ach	Acetylcholine
AChE	Acetylcholinesterase
ADI	Acceptable Daily Intake
Ai	active ingredient
ANOVA	Analysis of Variance
APHI	Action Pre-harvest Interval
ARfD	Acute Reference Dose
CAC	Codex Alimentarius Commission
CAN	Calcium Ammonium Nitrate
Ct	Concentration at time t(days)
CT ₅₀	Half-life Time (50%) for clearance of bioaccumulation
CV	Coefficient of Variation
DAP	Diammonium Phosphate
DBM	Doible Blind Maitenance
dMRM	Dynamic Multiple Reaction Monitoring
EC	Emulsifiable Concentrate
EDCs	Endocrine Disruptor Chemicals
EDI	Estimated Daily Intake
ESI	Electron Spray Ionization
EU	European Union
FAO	Food and Agriculture Organization
FOCUS	Forum for the Co-ordination of pesticide fate models and their USE
GAP	Good Agricultural Practices
GC-MS	Gas Chromatography – Mass Spectrometry

GC-MS/MS	Gas Chromatography tandem Mass Spectrometry
GOK	Government of Kenya
GPAP	Good Pesticide Application Practices
Ha	Hectare
HACCP	Hazard Analysis and Critical Control Points
HCDA	Horticultural Crops Development Authority
HHPs	Highly Hazardous Pesticides
HPLC	High Performance Liquid Chromatography
HRI	Health Risk Index
HRI _A	Health Risk Index for Adults
HRI _C	Health Risk Index for Children
IPM	Integrated Pest Management
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
KEPHIS	Kenya Plant Health Inspectorate Services
KOSFIP	Kimira - Oluch Smallholder Farmers Improvement Project
LC	Liquid Chromatography
LC- MS	LC- MS – Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
LD ₅₀	Lethal Dose (50%)
LOD	Limits of Detection
LOQ	Limits of Quantification
LSD	Least Significant Differences
MDGS	Millenium Development Goals
MeCN	Methyl cyanide (Acetonitrile)
MoALF	Ministry of Agriculture, Livestock and Fisheries

MRL	Maximum Residue Limit
MT	Metric tonnes
n.d	no date
N-P-K	Nitrogen – Phosphorus - Pottasium
NRC	National Resesrch Council (Cancer)
OECD	Organization for Economic Co-operation and Development
PAP	Post Application Period
PCPB	Pests Control Products Board
PHI	Pre-harvest Interval
PMRA	Pest Management Regulatory Authority
POPS	Persistent Organic Pollutants
Ppb	Parts per billion
PPE	Personal Protective Equipment
Ppm	Parts per million
QuEChERS	Quick Easy Cheap Effective Rugged and Safe
RCB	Randomized Complete Blocks
RCBD	Randomized Complete Block Design
REI	Re-entry Interval
RL ₅₀	Residual Level (50%)
Rpm	Revolutions per minute
S/N	Sound – to - Noise
SAS	Statistical Analysis Systems
SDGs	Sustainable Development Goals
SDS	Safety Data Sheets
SOP	Standard Operating Procedures

SP – RCBD	Split Plot – Randomized Complete Block Design
SPE	Solid Phase Extraction
tAPHI	Action Pre-harvest Interval at time t (days)
TE	Trace Elements
UN	United Nations
UNEP	United Nations Environment Programme
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UV	Ultra-Violet
WHO	World Health Organization of the United Nations
WG	Water-dispersible Granules
WP	Wettable Powder

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Agriculture provides livelihood to over 80% of the world population and equally caters directly and indirectly for about 80% of employment (Njeru, 2017). In most developing countries, agriculture is the backbone of the economies (Samoei & Kipchoge, 2021). In Kenya, agriculture contributes about 30% of the national Gross Domestic Product (GDP) (Central Intelligence Agency (CIA), 2014; Njeru, 2017), majorly driven by the horticultural sector (Samoei & Kipchoge, 2021). The significant contribution on the GDP is a consequence of Kenya government's strategy to revitalize agriculture, especially horticulture, with the aim of improving food security and a shift from subsistence to commercialized agriculture (Government of Kenya, 2013; Kangai *et al.*, 2011). Subsequently, the use of synthetic pesticides for the control of pests and diseases that would otherwise lower quality and yield on horticultural crops have increased with demand for the produce (Marete *et al.*, 2021; Ochilo *et al.*, 2019).

Environmental pollution by synthetic pesticides has been one of the world's leading challenges to the realization of good health and well being sustainable development goals (SDGs) of the United Nations (FAO, 2018b; UN, 2017). In addition, the effects may be a hindrance to the African Union Commission (AUC) 2063 agenda of environmentally sustainable and climate resilient green economies and communities (African Union Commission, 2015). Although pesticides are required to optimize food production (Alexandratos & Bruinsma, 2012; Sarkar *et al.*, 2021), their residues, magnified by malpractices in their use, are ubiquitous contaminants in the environment (Botwe, 2007; Hanson *et al.*, 2007; Kiwango *et al.*, 2018). The residues pose serious to fatal health hazards to non-target organisms through inhalation, contact and ingestion of contaminated food stuffs (Francisco, 2011; Juraske *et al.*, 2009). Approximately

30% (based on mass) of human food has been of vegetable origin, mostly consumed raw or semi-processed. Vegetables treated with pesticides are therefore likely to be a source of pesticide residue to human beings more than other food groups (Claeys *et al.*, 2011) given that their production involves use of various pesticides. Since ingestion of contaminated foodstuffs is the major exposure route (WHO, 2015), it is necessary that vegetables treated with pesticides during production are evaluated for pesticide residue safety levels to protect the consumers against food safety hazards and risks.

Brassica oleracea var. acephala, commonly called kale and “*Sukumawiki*” (Sarah & Maina, 2008), is a cruciferous vegetable belonging to the genus *Brassica*, species *Brassica oleracea*, group *Acephala* (MoALF/SHEPPLUS, 2019; Šamec *et al.*, 2019). It is grown in many parts of the world (Šamec *et al.*, 2019; USDA, 2014) as food and for its numerous health benefiting polyphenolic flavonoid compounds that minimize the risk of degenerative diseases like cancer (Francisco, 2011; Rosa & Heany, 1996). However, *Brassica oleracea var. acephala* cultivation involves application of pesticides for the management of pests and diseases that attack the roots and foliage (FAO, 2010a; Sarah & Maina, 2008; Seif & Nyambo, 2013). In Kenya, diazinon (*O,O*-diethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate) is one of the broadspectrum pesticides registered for use in *Brassica oleracea var. acephala* production (Pest Control Products Board, 2018). Diazinon is expected to undergo dissipation through chemical processes such as hydrolysis and other forms of physical transformations including wash-off from the leaves. It is expected that the total dissipation effect may reduce diazinon residues to levels below the Codex maximum residue limit (MRL) of 0.05 mg/Kg when diazinon is applied according to good agricultural practices (GAP). Due to changing environmental conditions, malpractices in use of the pesticides and *Brassica oleracea var. acephala*-specific physiological and phenological characteristics, diazinon may persist as residues on the vegetable for a long time (Aggarwal *et al.*, 2013; Freed *et al.*, 1979;

Kouloumbos *et al.*, 2003). However, it is not clear whether the residues remain on the *Brassica oleracea var. acephala* after harvest at levels that may present health risks to consumers.

Kimira-Oluch Smallholder Farmers Improvement Project (KOSFIP) is an irrigation project located in Homa Bay County in the Republic of Kenya. The ecological zone of the KOSFIP area is characterized by hot and humid climatic conditions with scanty rainfall of high variability in duration and amounts (Government of Kenya & African Development Fund, 2006; Jaetzold *et al.*, 2009). High relative humidity of the location could be as a result of the influence of Lake Victoria and the irrigation water channels of the project. These conditions promote rapid spread of vegetable pests and diseases (Cilas *et al.*, 2016; Nurhayati, 2011) which the smallholder farmers may manage by use of synthetic pesticides. Though diazinon has been used by the smallholder farmers of *Brassica oleracea var. acephala* at KOSFIP, the long pre-harvest interval (PHI) of 12 days (Pest Control Products Board, 2018) may not be observed by the farmers characterized by rampant pesticide use malpractices (Kiwango *et al.*, 2018). Consequently, the diazinon residues in *Brassica oleracea var. acephala* grown in KOSFIP could be above Codex MRL. Unfortunately, farm and market gate basket screening of diazinon residues in the produce at KOSFIP has not been done and it is not documented whether diazinon residues may be present in the farm-gate *Brassica oleracea var. acephala* vegetables at levels that might be a health risk to the consumers.

Survey and monitoring studies on farm and market gate baskets in diverse geographical locations have confirmed that pesticide residues including diazinon were present at inappropriate levels in different types of vegetables (Chen *et al.*, 2011; Hanson *et al.*, 2007; Prodhan *et al.*, 2018; Uysal-Pala & Bilisi, 2006). Since pesticide residue levels in fruits and vegetables primarily obey the rate of application proportionality principle (MacLachian & Hamilton, 2011; Sadlo, 2000), one of the suspect malpractices that may be associated with inappropriate levels has been disregard of the label rate of application (Egyptian Ministry for

Agriculture and Land Reclamation, 2017; Kiwango *et al.*, 2018). Residue levels are likely to change with rate of application (Cabras *et al.*, 1985; Cheah, 1985; Kabir *et al.*, 1970; Prodhan *et al.*, 2018). With heavy pests and diseases infestations, farmers are likely to use higher rates of diazinon (Kiwango *et al.*, 2018). The 12 days PHI may therefore be inappropriate for the higher doses. Consequently, residues of diazinon used at KOSFIP area may be at levels above the Codex MRLs. Since no vegetable-pesticide baseline trials have been done in the KOSFIP area, it has not been evaluated how the rate of application of diazinon influences levels of diazinon residues in *Brassica oleracea var. acephala*.

Pesticide dissipation rates and half-lives have been reported to vary across plant species and phenological stages (Cabras *et al.*, 1985, 1988, 1990; Valverde-Garcia *et al.*, 1993), physicochemical properties of pesticides and environmental conditions (Jacobsen *et al.*, 2015). The variations determine the setting of label pre-harvest intervals and maximum residue limits (MRLs) (Fantke & Juraske, 2013). Dissipation over a post application period may be due to chemical or microbial degradation (Hoagland *et al.*, 2000; Zablotowicz *et al.*, 2005) and growth dilution (Zongmao & Haibin, 1988). In addition, volatilization from plant surfaces (Karthika & Muraleedharan, 2009; Rosendahl *et al.*, 2009) and degradation through hydrolysis processes are influenced by multiple environmental factors including temperature and humidity (Pérez *et al.*, 2013). Since the label pre-harvest intervals (PHI) may not have been decided in areas similar to KOSFIP (Hanafi *et al.*, 2018), it is therefore not determined if the recommended PHIs are suitable for KOSFIP. There is an information gap as to how the levels of diazinon residues may vary with post application durations of the pesticide in *Brassica oleracea var. acephala*.

1.2 Statement of the problem

Pests and diseases are major problems in crop production. For *Brassica oleracea var. acephala* production at Kimira-Oluch Smallholder Farmers Improvement Project (KOSFIP) in Homa Bay County of Kenya, diazinon is extensively used to control pests. Despite the widespread use of diazinon, there has not been any evaluation of diazinon residues levels or assessment of health risks the residual levels may pose. Farmers use varying rates of the diazinon on *Brassica oleracea var. acephala* in the project area. Some use the recommended rates as specified on the packets. Whether these rates which were evaluated at different sites are appropriate for KOSFIP is not documented. Farmers usually use higher rates than the recommended rates if they perceive that the level of insect infestation is high. Despite the high rates, the farmers using GAP as recommended on the pesticide labels observe the recommended PHI. Finally, it is not documented if use of higher rates of diazinon will require longer PHI.

1.3 Objectives of the study

1.3.1 General objective.

To evaluate the residue levels of diazinon in farm-gate vegetables and how some selected application conditions influence the levels in *Brassica oleracea var. acephala* in Kimira – Oluch Smallholder Farm Improvement Project (KOSFIP) area.

1.3.2 Specific objectives.

The specific objectives of the study are to:

- a) Quantify the levels of diazinon residues in the farm- gate baskets of *Brassica oleracea var. acephala* from the KOSFIP area of Homa Bay County for health risk assessment
- b) Determine the influence of rate of application of diazinon on residual levels of diazinon in *Brassica oleracea var. acephala* of KOSFIP area at the recommended pre-harvest interval

- c) Determine the effect of post application harvesting duration on residual levels of diazinon in *Brassica oleracea var. acephala* of the KOSFIP area at different rates of application.

1.4 Research question and null hypothesis (H₀)

The study addressed the following research questions and null hypotheses:

- a) Research questions
- i) Do the levels of diazinon residues in farm-gate *Brassica oleracea var. acephala* of KOSFIP meet the Codex recommended maximum residue levels?
 - ii) Do the health risk indices (HRI) resulting from residual diazinon levels in farm gate *Brassica oleracea var. acephala* of KOSFIP fall within tolerable safety ratios?
- b) Null hypothesis (H₀)
- i) At the recommended pre-harvest interval (PHI), residue levels of diazinon in *Brassica oleracea var. acephala* of the KOSFIP area do not vary with rates of applications
 - ii) At different application rates, there is no variation in the residue levels of diazinon in *Brassica oleracea var. acephala* of the KOSFIP area with post application durations

1.5 Justification of the study

The established dietary exposure of consumers to diazinon through *Brassica oleracea var. acephala* would have resulted to increased chronic and acute health risks. Equally, the uncontrolled diazinon application conditions and practices would have increased environmental contamination levels and consequent deleterious effects on ecological balance at KOSFIP. Consequently, the production of *Brassica oleracea var. acephala* under the study

recommendations will be important in ending hunger, achieving food security and improved nutrition while promoting sustainable agriculture as proposed by the sustainable development goals of the United Nations, the African Union agenda 2063 and Kenya vision 2030.

1.6 Assumptions of the study

The study was conducted under the assumption that washing of the vegetables may not pointedly reduce the residual levels and subsequent risks due to application of diazinon on *Brassica oleracea var. acephala* at the study area. In addition, it was assumed that the term diazinon residues refer to the undissipated parent molecule at the time of harvesting as used in determination of compliance to maximum residue levels (MRL) and dietary intake definitions, and not to its metabolites, transformation products or impurities arising from its use on the vegetable. Finally, the study assumed that the formulation Diazol 60 EC used in the trials was adequately stabilized by manufacturers and stored under appropriate conditions by distributors and farmers so as to eliminate chances of self decomposition to toxic degradation products.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Pests are organisms considered by humans as a threat to human life, crops, animals or property (Ash, 2003). Pesticides have been used to control or eradicate the pests (Okolle et al., 2014; Pimentel *et al.*, 2013; Seif & Nyambo, 2013; van den Berg *et al.*, 2020; WHO, 2015; Yadav *et al.*, 2015). While non-use of synthetic pesticides would lead to food insecurity due to food losses and public health deterioration resulting from disease vectors (Okolle *et al.*, 2014; Pimentel *et al.*, 2013; Seif & Nyambo, 2013), routine application of the otherwise toxic tools of convenience have resulted in environmental pollution and contamination of food stuffs with multiple human health challenges (USEPA, 2004). Consequently, sustained monitoring of residue levels in food crops and environmental matrices should be done to mitigate health risks (Michaela & Stanescu, 2014; USEPA, 2004). It is widely believed that unregulated use of pesticides may be responsible for inappropriate residue levels in food crops (Mahmood *et al.*, 2016) and increasing disease vector resistances to the available pesticides (WHO & FAO, 2019). As a result, attainment of the sustainable development goals (SDGs) of the United Nations (FAO, 2018b), the agenda 2063 of the African Union Commission (African Union Commission, 2015) and the Kenyan vision 2030 (Government of Kenya, 2017) may not be achieved.

In this study, the use of diazinon in the production of *Brassica oleracea var. acephala* at the Kimira – Oluch Smallholder Farmers Improvement Project (KOSFIP) area of Homa Bay, Kenya has been evaluated for residues of diazinon. In addition, the influence of label application conditions of rates and post application periods have been determined so as to provide baseline information for the establishment of good agricultural practices for sustainable production of *Brassica oleracea var. acephala* in the study area.

2.2 Pesticides and their uses

2.2.1 Pesticides.

Pesticides are chemical or biological substances intended for controlling or killing pests during production and storage of agricultural crops, and in the health sector for control of disease vectors (WHO, 2015; Yadav *et al.*, 2015). When pesticides are applied in an environment, the target pests may be attracted to the pesticide with consequent destruction or changes in the physiological characteristics of the pest or repelled from the site of application (Mahmood *et al.*, 2016). Ultimately, harmful effects of pests on food crops will have been mitigated. Effects of use of pesticides on target pests are dependent on duration and levels of exposure, toxicity levels and timing of application (FAO, 2012b; Schmolke *et al.*, 2010). While the usefulness of pesticides have been primed on the toxicological and repellent effects on pests and disease vectors, the effects have to a larger extent been drawn-out to the environment and non-target organisms including humans (Sarkar *et al.*, 2021; Yadav & Devi, 2017). Some of the emerging toxicological interactions in the environmental matrices have associated pesticides to “mutagenicity, carcinogenicity, hormone modulant effects of environmental endocrine disruptor chemicals (EDCs), immunomodulant effects”(Székács *et al.*, 2015). Subsequently, residues of pesticides frequently found in food commodities and the environment may be ecologically deleterious (Mahmood *et al.*, 2016). Pesticide use, therefore, raises environmental sustainability concerns that increase with usage.

2.2.2 Classifications, types and uses of pesticides.

Various classes and types of pesticides are produced and used globally based on guidelines provided by World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations (WHO, 2009; Yadav & Devi, 2017). The classes and types (Appendix A) are available in the market as formulations fitting the biology of target pests (Appendix B) and the physical and chemical characteristics of pesticide active ingredients (ai) (FAO, 2012b).

Pesticide formulations are designed to maximise efficacy while minimizing potential risks to the environment and to the applicators (FAO, 2012b; WHO, 2009). All classes and types of pesticides (Appendix A) exhibit varying levels of activity requiring sustainable use through proper good agricultural practices (GAPs) (Maksymiv, 2015; Özkara *et al.*, 2016). However, the adoption and implementation of GAPs has not been achieved globally and risks due to pesticide use have not been contained.

2.2.3 Pesticide usage in agriculture.

Pesticide use in agriculture is widely accepted (Neha & Praveen, 2014). The acceptance has been based on pesticide effectiveness and reliability in the management of pests and diseases that threaten food production. As a result, an estimated 25% of the earth's terrestrial surface is under crop production and involving over 67% of the global population. Global pesticide usage report indicated a total of about 3 million metric tons per year (Atwood & Paisley-Jones, 2017). Herbicides comprised over 50%, followed by fumigants, insecticides and fungicides, respectively, of the pesticides. A global average pesticide usage cost-benefit ratio for the period 2010 – 2014 was at 0.645 g pesticide use for each kilogram crop produced and a mean annual usage of 2.784 Kg/ha. However, the resultant agricultural pollution due to global usage of pesticides is not clear.

2.2.4 Pesticide usage in developed nations.

Pesticide use statistics in developed countries has been reliably documented and is widely guided by legislative directives and regulations (Sharma *et al.*, 2019). The regulations maintain registered pesticides in the market and monitor the maximum residue levels (MRLs) in foods and feeds, plants and animals (Lobin *et al.*, 2017). Among the developed nations, herbicides are the most popular pesticide and major environmental and food contaminant because they are an alternative to high cost of human labour in agriculture (Karasali *et al.*, 2002; Kristoffersen

et al., 2008). Stringent regulations on pesticide usage supported by strong political will and public interest in these countries have supported the lowering of pesticide applications and effect on the environment (Kristoffersen *et al.*, 2008). As a result, pesticide safety levels and health risk assessments for the food crops are frequently monitored. In addition, data on pesticide use and residue levels in various food crops are available and useful in reviews of related legislations promoting environmental sustainability (WHO & FAO, 2019). Thus, pesticide residues hardly exceed the Codex and European Union acceptable levels in the environmental matrices and foods.

2.2.5 Pesticide usage in developing countries.

The economies of developing countries are highly dependent on agriculture (Neha & Praveen, 2014). As a result, about 20% of the global pesticide usage share has been distributed to developing countries (Sarkar *et al.*, 2021; Sharma *et al.*, 2019). Even though pesticide usage statistics for these countries are not easily obtainable (WHO & FAO, 2019), the demand for pesticides has been on the increase due to increasing populations and consequent demand for food (Neha & Praveen, 2014). Unlike the pesticide use conditions in developed nations, most developing countries lack stringent regulations, strong political will and public interests that favour sustainable safe use of pesticides (Ecobichon, 2001; Kristoffersen *et al.*, 2008). In addition, pesticide dietary exposure levels are scarcely determined and health risk assessments of food produce in the developing countries are rarely done.

2.2.6 Challenges arising from pesticide use.

Whereas global adoption and dependence on synthetic pesticides have been correlated with rise in food security and control and eradication of disease vectors (Neha & Praveen, 2014), sustainability of safe pesticide use has experienced numerous challenges (FAO, 2018b). The challenges emanate from unreconciled intercountry and regional gaps in pesticide registration

and management legislations (WHO & FAO, 2019). The gaps are occasioned by inadequate pesticide residue monitoring guidelines and infrastructure (FAO & WHO, 2013; WHO, 2011), unregulated pesticide markets (Boone *et al.*, 2014; WHO & FAO, 2010), low adoption of integrated pest management (IPM) alternatives (FAO, 2012b), and some conflicting national and regional pesticide safety level regulatory standards that contradict Codex Alimentarius Commission guidelines (WHO & FAO, 2019). In addition, inadequate logistical infrastructure for handling highly hazardous pesticides (HHPs) (WHO & FAO, 2017) and inconsistencies in data compilation on environmental contamination and pesticide residue monitoring also hinder desired sustainability goals of the United Nations Organization (FAO & WHO, 2019). Lastly, inadequacies have been reported in the management of pesticide resistance (FAO, 2012b), transport, storage and disposal of pesticides (FAO & WHO, 2014), quality controls in applicator equipment, and training and certification of applicators on use of personal protective equipment (PPEs) (WHO & FAO, 2019). As a result of these challenges, pollution problems associated with pesticide use in agriculture and disease vector management remain a threat to environmental safety and wellness.

2.2.7 Problems arising from pesticide use.

The use of synthetic pesticides in agriculture and disease vector management has been linked to pollution and poisoning problems in environmental matrices (Michaela & Stanescu, 2014; Nnamonu & Onyekutu, 2015). Regardless of the system of production (Uysal-Pala & Bilisi, 2006), food crops treated with pesticides have been found to contain some residues of legacy and currently used pesticides (Hanson *et al.*, 2007; Hussain *et al.*, 2002). Pesticide residues are chemical contaminants with the potential to reduce nutritional value of foods and cause degenerative diseases (Aktar *et al.*, 2008; FAO & WHO, 2006; Pujeri *et al.*, 2016). Increasing pesticide use in agriculture has led to pest resistance to available pesticides (FAO, 2012b; WHO & FAO, 2019), contamination of surface and ground water sources (Aydinalp & Porca, 2004;

Srivastava *et al.*, 2019; Székács *et al.*, 2015), poisoning and deleterious effects on non-target living organisms including humans, endangered species, earthworms and pollinators (Aktar *et al.*, 2008; WHO & FAO, 2019; Yadav & Devi, 2017). While the residual pesticides in the environmental matrices leading to toxicological problems may be expected to dissipate with time, some are persistent and may bioaccumulate and bioconcentrate causing health hazards even in very low concentrations (Abdelgadirand & Adam, 2011; Gebremariam *et al.*, 2012; Zidan, 2009). However, data on dissipation parameters are scarce and may also vary with changing environmental conditions.

2.2.8 Pesticides and sustainable development

The concept of sustainable development launched by the Brundtland World Commission on Environment and Development (1987) is understood as “development that meets the needs of the present without compromising the ability of the future generations to meet their own needs” (United Nations General Assembly, 1987). The challenges and problems associated with pesticide uses, especially in developing countries, point to an urgent need to embrace sustainable management of pests (Sarkar *et al.*, 2021). While pests and disease vector control are major strategies in addressing sustainable development goals (SDGs) with respect to food security, improved nutrition, healthy lives and well-being for all, and sustainable agricultural production and consumption (African Union Commission, 2015; FAO, 2018b, 2018a; Government of Kenya, 2017; WHO & FAO, 2019), the ubiquitous pesticide residues in the global environment remains a threat to green economy. Consequently, environmental degradation, climate change and biodiversity losses resulting from pesticide use demand for control and regulatory measures to sustain the environment.

2.3 Control and regulations on pesticide use

In response to the challenges and problems associated with pesticide use in agriculture, and to advance green economy and sustainable development, the United Nations Organization controls and regulates the use of pesticides through international legislations (FAO & WHO, 2019). The legislations address registration, re-registration, harmonization and homologation of registered pesticides, and formulation and review of good agricultural practices.

2.3.1 Registration and harmonization of pesticides for use.

The World Health Organization and Food and Agriculture Organization of the United Nations Organization (UN) control the registration and reregistration of pesticides (FAO & WHO, 2016a). Joint meetings involving FAO and WHO experts in partnership with the Codex Alimentarius Commission regulate, approve, review and harmonize pesticide registrations and use standards as provided by each pesticides' safety data sheets (SDS) and labels (Hamilton *et al.*, 2017; Yamada, 2017; Yeung *et al.*, 2017). Some of the legislations often reviewed and harmonized include restrictions and bans for highly hazardous pesticides (Appendix C) guidelines on good agricultural practices (GAPs), maximum residue limits (MRLs), acceptable daily intake, acute reference doses and hazard analysis and critical control points (HACCP) procedures (Food Safety and Inspection Services, 1996). However, compliance to the set guidelines varies according to national legislations. Under weak surveillance and monitoring of agronomic activities, the food safety hazard levels become unpredictable and the risks to consumers may be lethal.

2.3.2 Good agricultural practices (GAPs).

Good Agricultural Practices (GAPs), commonly called FAO GAP, are policy guidelines targeting sustainable hands-on farming activities for the production of safe and quality foodstuff and other non-food agricultural products (FAO, 2007a, 2007c; Ojiewo *et al.*, 2013).

As a technological advancement strategy, the concept of GAPs has been developed and given a global outlook through national and regional adoptions (FAO, 2010b). From this viewpoint, each country is expected to adopt the FAO GAP as a response to the need for environmental responsibility (FAO & WHO, 2019; FAO, 2018b, 2018c). However, all farmers in the respective countries of the world may not be exposed to GAPs and the safety and environmental sustainability of produce from such countries remains doubtful.

Good pesticide application practices (GPAP) is a specific version of FAO GAP that customizes the international code of conduct on the safe use of pesticides for effective and reliable pest control” (FAO, 2010b, 2012a). GPAP guidelines discourage malpractices such as use of unregistered and banned pesticides, inappropriate dosages of registered pesticides, and disregard of label PHI (FAO, 2012b). In addition, GPAP discourages disregard for pesticide – crop combination, and use of a mix of pesticides in a single spray without consideration of their interactive effect on the crops and environment. The realization of the GPAP guidelines is also subject to national legislations and surveillance on farmer practices and is dependent on integrity, knowledge and skills by individual farmers (Kiwango *et al.*, 2018). In circumstances where farmer training and legislations are inadequate, the farm produce may be unsafe for human consumption and the extent of environmental pollution undefined.

2.3.3 Maximum residue limits.

Maximum residue limits (MRLs) are pesticide-specific health risk determinants defining the “maximum concentration of a pesticide residue (mg/Kg) that the Codex Alimentarius Commission (CAC) recommends as legally permitted in food commodities and animal feeds” (FAO & WHO, 2004). Since pesticides are inherently toxic and are known to undergo bioconcentration and bioaccumulation, no residue levels are entirely safe (Abdelgadirand & Adam, 2011; Zidan, 2009). For this reason, the maximum residue limits are set as low as possible to minimize effects on consumers.

2.3.4 Other pesticide residue regulatory parameters.

In addition to the CAC MRLs, food safety regulations are done through continuous assessment of dietary intakes of pesticide residues monitored using acceptable daily intake (ADI), estimated daily intake (EDI) and acute reference doses (ARfD) (Ecobichon, 2001; FAO & WHO, 2004; Nasreddine & Parent-Massin, 2002; Stenersen, 2004). The ADI, EDI and ARfD form part of pesticide registration, determined by field trials guidelines (OECD, 2011). CAC recommends frequent monitoring of levels of pesticide residues in foods and feeds. However, the international directives major on produce to international markets. Very little is known about levels of residues in locally produced and consumed food crops.

2.4 Pesticides and their uses in vegetable production

2.4.1 Vegetables.

Vegetables are a large commodity group of edible plants or plant parts which when consumed raw or processed provide essential human nutrition (Belitz *et al.*, 2004). Vegetables do not have common botanical features but generally share similarities in cultivation methods and pests and diseases that affect their growth and yields (Mike & Martin, 2009). The utilization of vegetables as human food has been part of man's cultural heritage and plays important roles in the customs, traditions and food culture of every household (Adedokun, 2017; Mensah *et al.*, 2008). Regular consumption of vegetables has been promoted (WHO, 2005) due to the beneficial biochemical and pharmacological compounds in vegetables that provide protective and curative effect on human health (Katerere *et al.*, 2012; Kris-Etherton *et al.*, 2002). The market and demand for vegetables is therefore insatiable. To ensure the quality and quantities, their production involves persistent and vigorous use of pesticides against pests and diseases (Wenjun *et al.*, 2011). As a consequence of pesticide use, it should not be assumed that any

portions of these vegetables are safe for human consumption unless the levels are checked against the MRL standards to minimize possible health risks.

2.4.2 Diversity of cultivated vegetables.

Several vegetable types and varieties of vegetables (Appendix D) are cultivated globally with minor variations in use according to cultures and customs. Europe, Americas and Asia have the broadest varieties of cultivated vegetables (De Cicco, 2016; Hong & Gruda, 2020). Similarly, Africa's list of cultivated indigenous and traditional leafy vegetables have been enriched by improved exotic vegetable species from the European, American and Asian continents (Abukutsa-Onyango, 2007; Akinola et al., 2020; Chweya, 1997; Mnzava, 1997). While indigenous African cultivated vegetables display multiple agronomic advantages over the exotic varieties (Abukutsa-Onyango, 2002; Maundu, 1997), they are slowly getting abandoned as a consequence of racial integration and migrations of peoples across the continents and pressure of evolving diversity in consumer preferences (Ojiewo *et al.*, 2013). Consequently, the improved varieties are competitively replacing the indigenous vegetables (Mike & Martin, 2009). On this premise, *Brassica oleracea var. acephala*, considered by a larger global population as a superfood, has steadily grown in popularity and production (Šamec *et al.*, 2019). In the East African region, the production of African leafy vegetables is declining, especially within the Lake Victoria Basin and giving way to *Brassica oleracea var. acephala* and other exotic vegetables (Government of Kenya, 2016) that require application of synthetic pesticides to sustain yields (Keatinge *et al.*, 2015). However, the synthetic pesticides used on these vegetables to sustain productivity are toxic and have potential for vegetable contamination and multiple health risks to consumers.

2.4.3 Constraints in vegetable production.

The major constraint experienced in vegetable production across the globe is pests infestations

(Phophi & Mafongoya, 2017). The pests are disease vectors and feed on the vegetables (Brown *et al.*, 2001). Pests, if not managed in time can cause up to 100% losses during severe infestation (Okolle *et al.*, 2014; Seif & Nyambo, 2013). In addition to pests, inadequate productive land, low rainfall, and high human population density also hinder optimum production of vegetables (Dixon *et al.*, 2001; FAO, 2007c, 2007a; Phophi & Mafongoya, 2017). The high population density reduces land available for farming but significantly increases demand for the vegetables. To meet the increasing demands for vegetables and the challenge of inadequate and irregular rainfall, irrigation of arid lands and application of pesticides have been adopted (Phophi & Mafongoya, 2017). However, irrigation increases humidity and rapid pests development thereby increasing the need to use pesticides (Abukutsa-Onyango, 2007; Nurhayati, 2011). While pesticides may be persistent and harmful to the environment (FAO, 2007b, 2007a, 2007c, 2010b, 2010a; WenJun *et al.*, 2011; WHO, 2015), the environmental sustainability of their use is based on good agricultural practices (GAP) and frequent monitoring of the residue levels at the farm-gate baskets (NRC, 1993).

2.4.4 Management of pests in vegetable production.

Pesticide use in vegetable farming has been necessitated by the increasing number of plant pests (insects and mites), pathogens and weeds which destroy vegetables (Pimentel, 2009). About 4.6 million tonnes of chemical pesticides are annually sprayed into the environment to manage these pests and associated diseases in farms (Wenjun *et al.*, 2011). In view of the world's limited and diminishing croplands and the growing population (W. Zhang, 2008; W. Zhang *et al.*, 2006), pesticide use in the management of pests has become a key component of sustainable vegetable farming and is indispensable in agricultural production (Nnamonu & Onekutu, 2015; Zhang *et al.*, 2007; Zhang, 2008; Zhang, 2009). While the pesticides display varied physico-chemical environmental fate properties which may lead to varying environmental exposure levels, data on dissipation patterns and persistence in vegetable

matrices under varied environmental conditions may be limited or non-existent. Consequently, implementation of GAPs may not yield desired results in pesticide safety levels.

2.4.5 Pesticide dissipation and environmental exposure.

Pesticide dissipation is the sum total of all physical, chemical and biochemical processes which reduce the concentration of a pesticide on or in an environmental matrix soon after application (Deister & Crosby, 1999; Fantke & Juraske, 2013; Seiber & Kleinschmidt, 2010). The rate of pesticide dissipation is defined by the dissipation half-life (RL₅₀) values which estimate the time taken to reduce the initial pesticide residue concentration (C₀) level to half (Lewis & Tzilivakis, 2017). RL₅₀ values are determined during controlled field trials. Since dissipation rates influence availability, exposure and subsequent toxicity of the residual pesticide in an environmental matrix (Aggarwal *et al.*, 2013), the RL₅₀ values have been used in the determination of risk assessment of pesticides to target and non-target organisms (Mueller & Senseman, 2015). Most pesticide dissipation patterns have been fitted to the pseudo-first-order kinetics model (FOCUS, 2006; González *et al.*, 2001; Hoskins, 1996) given by the Langmuir-Hinshelwood function:

$$C_t = C_0 e^{-kt} \dots\dots\dots (1)$$

Where

C_t is the residue concentration in mg/Kg at a time t in days post application

C₀ is the initial concentration of the residues in mg/Kg at t=0 days

k is a constant rate of pesticide dissipation efficiency per day

t is the post application period in days after the pesticide application.

From equation (1), k can be determined by the function:

$$k = [\ln (C_0) - \ln (C_t)]/t \dots\dots\dots (2)$$

For varied pesticide application conditions and matrices, the constant of dissipation efficiency (k) is unique and determines the half-life of the pesticide (Fantke & Juraske, 2013). From the first-order kinetic regression model, the dissipation half-life, RL_{50} ($t_{1/2}$) equation derived from the general model (equation 1) is given by (Hoskins, 1996; Juraske *et al.*, 2008):

$$t_{1/2} = [\ln (2)]/k \dots\dots\dots (3)$$

Consequently, the corresponding action pre-harvest intervals (t_{APHI}) at which a specific residual level of a pesticide may be realized for each dissipation regression model fitted into the pseudo-first-order-kinetics may be determined by the expression:

$$t_{APHI} = [\ln (C_t) - \ln (C_o)]/k \dots\dots\dots (4)$$

While most pesticide – plant species combinations’ dissipation studies follow first-order-kinetics, there are few exceptions of dissipation patterns that follow non-first-order models (Appendix E) (Fantke & Juraske, 2013). Non-first–order models have been reported for various environmental matrices with all chemical structure classes of pesticides (carbamates, organochlorines, pyrethrins, pyrethroids, organophosphates and neonicotinoids). Regardless of the order of dissipation kinetics, safe post application periods corresponding to residual amounts \leq Codex MRLs in a matrix should be established to minimize health risk effects (Lewis *et al.*, 2016).

2.4.6 Dissipation of pesticides in vegetables.

Variability in organophosphate pesticides dissipation rates have been reported between pesticides, plant species and environmental conditions resulting in dissipation half-lives ranging from a minimum of 0.6 and a maximum of 29 days (Fantke & Juraske, 2013). The variations have been associated with pesticide decomposition through chemical or microbial degradation (Magri & Haith, 2009) aided by oxidative, reductive and hydrolytic mechanisms (Hoagland *et al.*, 2000) or through conjugative processes and bound residues (Van Eerd *et al.*,

2003; Zablotowicz *et al.*, 2005). As a result of microbial degradation, some pesticide metabolites have been absorbed by degrading microorganisms as energy pools and growth substrates (Magri & Haith, 2009).

Plant surface-related aspects of dissipation such as growth dilution (Zongmao & Haibin, 1988) and volatilization from plant surfaces (Fenoll *et al.*, 2008, 2009; Grover *et al.*, 1994; Guth *et al.*, 2004; Karthika & Muraleedharan, 2009; Nash *et al.*, 1977; Rosendahl *et al.*, 2009; Stork *et al.*, 1998) also result in apparent elimination of pesticide residues. Growth dilution increases with plant growth rate and pesticide stability on plant matrices while volatilization is based on pesticide vapor pressure. Volatilization processes increase with increases in solar irradiation and temperature but reduces with increase in humidity (Karthika & Muraleedharan, 2009; Sundaram, 1997). Acidity of the vegetable matrix is another key factor that influences dissipation on plant surfaces. Acidic samples catalyze hydrolytic degradation and enhance oxidative and hydrolytic processes (Athanasopoulos *et al.*, 2000).

Environmental conditions of irradiation intensity (Burrows *et al.*, 2002; Katagi, 2004) temperature (Katagi, 2004; Marin *et al.*, 2003; Stenersen, 2004; Willis & McDowell, 1987), precipitation (Fisher *et al.*, 2002) and spatial variabilities (Bending *et al.*, 2006; Bennett *et al.*, 1994; Dubus *et al.*, 2003) also influence dissipation. While the contributing factors of dissipation may be different for each vegetable species and varieties and environmental conditions, there have been tendencies to extrapolate findings of trial studies to various minor vegetables and environments (Park *et al.*, 2009). Park *et al.*, (2009) extended findings to varieties of *Brassica* genus such as *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *italica*, *Brassica oleracea* var. *acephala* and *Brassica oleracea* var. *botrytis*, which all differ phenologically and morphologically. However, little regard has been taken of the effect of such differences on rate of pesticide dissipation across varieties.

2.5 Pesticides and *Brassica oleracea* var. *acephala* production

2.5.1 *Brassica oleracea* var. *acephala*.

Brassica oleracea var. *acephala* (Figure 2.5.1) is a cruciferous vegetable belonging to the genus *Brassica*, species *Brassica oleracea*, group *acephala* (Dhaliwal, 2017; Šamec *et al.*, 2019). It has been used as a food crop since about 2000 BC, and is among the oldest vegetables in human history (Nieuwhof, 1969; USDA, 2014; Youdin & Joseph, 2001). Its ability to tolerate harsh climates and unpredictable weather fluctuations made it popular with farmers across the globe (USDA, 2014).



Figure 2.5.1: Photograph of *Brassica oleracea* var. *acephala* at KOSFIP experimental plot (3 weeks after transplanting) (Personal collection, February, 2020).

At present, *Brassica oleracea* var. *acephala* is grown in Brazil, Portugal, United States of America, Bosnia and Herzegovina, Croatia, Spain, India and many parts of Africa including Kenya. Due to the increasing global population, decreasing size of arable land and discovery of the nutritional value of the vegetable, its demand in many parts of the world is on the increase but is largely unmet due to pests and diseases infestations (Šamec *et al.*, 2019). Use of synthetic pesticides is widely practiced to eradicate the pests. Unfortunately, management of pests and diseases using synthetic pesticides leads to pesticide residue contamination of the vegetable

and causes environmental pollution.

2.5.2 *Brassica oleracea* var. *acephala* production trends in Kenya.

Brassica oleracea var. *acephala*, commonly called “Sukumawiki”, is grown in all the 47 counties of Kenya (Appendix F) and is the most consumed vegetable (Government of Kenya, 2016; Mutai *et al.*, 2016). Its annual production has been increasing for the last decade (Table 2.5.1) but for the constraints highlighted in Section 2.4.3. The growth is associated with high demand and increasing pesticide imports and use (Appendix G). Consequently, health risks effects of the pesticides on the environment and as residues in the vegetables may be increasing.

Table 2.5.1: Production trends showing area, quantity and monetary value of *Brassica oleracea var. acephala* in Kenya between 2004 and 2016.

Production Parameter	Years of production compared		
	2004	2014	2016
Area (Ha)	28,250	24,422	32,347
Quantity of produce (MT)	423,750	348,637	478,121
Value of produce (KShs)	4,237,500	4,844,000	6,954,782,771

Ha: hectares; MT: metric tonnes; KShs: Kenya Shillings.

Notes: The decline in quantity of produce between 2004 and 2014 was associated to multiple pests and diseases. Increased production between 2014 and 2016 was associated to adoption of multiple pesticides in the control of pests and diseases. Adapted from GoK (2016)

2.5.3 Production of *Brassica oleracea var. acephala* in Homa Bay County, Kenya.

Previously, smallholder farmers in Homa Bay County practiced traditional rain-fed seasonal farming with consequent losses of *Brassica oleracea var. acephala* due to pests and unreliable weather conditions (MoALF, 2016). Currently, some farmers have embraced irrigation and pesticide use technologies to produce *Brassica oleracea var. acephala* for local consumption. The imports of the vegetable from the neighboring Kericho, Kisii and Nyamira counties have correspondingly declined (Government of Kenya, 2014). Consequently, Homa Bay County registered a 3.2% of the national produce (Appendix F) of *Brassica oleracea var. acephala* in 2016 (Government of Kenya, 2016). KOSFIP area is the highest producer of *Brassica oleracea var. acephala* per unit area in Homa Bay County (MoALF, 2016). From the onset of the KOSFIP project in 2003 to the first appraisal in 2006, *Brassica oleracea var. acephala* production increased by 886% (Government of Kenya & African Development Fund, 2006).

However, the pesticide safety levels of the produce are unknown.

2.5.4 Production of *Brassica oleracea var. acephala* in Kimira - Oluch Smallholder Farmers Improvement Project area.

Kimira-Oluch Smallholder Farmers Improvement Project (KOSFIP) is an irrigation project initiated by the Government of Kenya to address the then Millennium Development Goals (MDGs) of the United Nations Organization (Government of Kenya, 2016). The project is located in Homa Bay County in the Republic of Kenya and was jointly funded by the Government of Kenya and the African Development Fund. It is strategically located to exploit the waters of River Kibuon and River Tende, which cut across the project area from the Gusii highlands. The river waters are used for irrigation of arable land for competitive smallholder agricultural activities. The project aimed at alleviating high poverty incidence occasioned by high population density, unreliable rainfall and frequent crop failures. The ecological zone of KOSFIP area is characterized by hot and humid climatic conditions with scanty rainfall of high variability in duration and amounts (Government of Kenya & African Development Fund, 2006; Jaetzold *et al.*, 2009). High relative humidity of the location is experienced as an influence of nearby Lake Victoria and the irrigation water channels. These conditions promote rapid spread of vegetable pests and diseases (Cilas *et al.*, 2016; Nurhayati, 2011). Though the farmers in the project area use pesticides to protect the vegetables, characteristic inadequacies of developing countries with respect to GAP (FAO, 2007c, 2007a, 2007b, 2010b) may be rampant. Due to these inadequacies, inconsistencies in pesticide dosages and non-observance of pre-harvest intervals (Kiwango *et al.*, 2018) may be common. Such malpractices (Adesuyi *et al.*, 2018; Kiwango *et al.*, 2018) may result in inappropriate residue levels in the vegetable produce at the farm-gate baskets. Indeed, pesticide safety levels in the farm-gate produce

remains unknown.

2.5.5 Pesticides used in *Brassica oleracea var. acephala* production.

Some of the common pests that affect *Brassica oleracea var. acephala* include aphids, thrips, red spider mites, whiteflies, Diamond black moth, fruit flies, fruit worms, locusts and grasshoppers (Michalik, 1994; Seif & Nyambo, 2013). Under increased leaf wetness and wide variations in day and night temperatures, the pests transmit fungal, bacterial and viral diseases and pathogens (Colhoun, 1973; Elad & Pertot, 2014; Huber & Gillespie, 1992). The pests and subsequent diseases are managed by use of insecticides and fungicides (Nyakundi *et al.*, 2010; Seif & Nyambo, 2013). Since organochlorines were declared as persistent organic pollutants (POPs) during the Stockholm Convention in 2001 and banned for use in vegetable production, the presumably less hazardous and degradable insecticide and fungicide pyrethrins, pyrethroids, carbamates, organophosphates and neonicotinoids have been registered for use in vegetables including *Brassica oleracea var. acephala* (Table 2.5.3) (Aryal *et al.*, 2016; Kolani *et al.*, 2016; Maksymiv, 2015; Palmquist *et al.*, 2012). From the list of registered pesticides (Table 2.5.3), diazinon stands out uniquely as the only organophosphate registered for use in *Brassica oleracea var. acephala* production. Nevertheless, diazinon use has controversies built around its effects on human health: effects which may increase with unregulated use in the environment (USAID-KAVES, 2014). Disregard of the recommended PHIs may lead to unacceptable residue levels with resultant exposure of the general population to health risks (Kiwango *et al.*, 2018). However, the levels of residual levels of these pesticides (Table 2.5.3) in locally consumed produce are hardly checked in Kenya. Consequently, there is need to establish data for safe use of pesticides in *Brassica oleracea var. acephala* produced at KOSFIP.

Table 2.5.5: Pesticides registered in Kenya for use in Brassica oleracea var. acephala production with corresponding target pests and recommended pre-harvest intervals.

Trade Name	Chemical class	Target pests	PHI
Alpha-cypermethrin	Pyrethroid Insecticide	Diamond Black Moth (DBM), aphids, thrips, cutworms, leaf miners, cabbage loopers	3
Bifenthrin	Pyrethroid insecticide	DBM, aphids, thrips, whiteflies, caterpillars	7
Cypermethrin	Pyrethroid Insecticide	leafminers, thrips, aphids, whiteflies, caterpillars	6
Deltamethrin	Pyrethroid Insecticide	DBM, leaf miners, thrips, aphids,	3
Diazinon	Organophosphate Insecticide	DBM, aphids, thrips, spider mites, whiteflies, mealy bugs, jacaranda bugs, scale insects, caterpillars, leaf beetles,	12
Imidacloprid	Neonicotinoid Insecticide	DBM, thrips, aphids, whiteflies,	7
Lambda-cyhalothrin	Pyrethroid Insecticide	DBM, aphids, cutworms, caterpillars, thrips, whiteflies	3
Metalaxyl-Mancozeb	Acylalanine/carbamate Fungicide	Downy mildew	7
Pyrethrins	Pyrethrin Insecticide	DBM, aphids, thrips, caterpillars, cabbage saw flies	1
Thiamethoxiam	Neonicotinoid Insecticide	aphids, thrips, whiteflies	7

Notes: Adapted from Pest Control Products Board (2018). Most of the pesticides are knockdown pyrethroids and neonicotinoids with recommended PHIs ranging between three and seven days. The broadspectrum organophosphate diazinon has the longest recommended PHI of 12 days with documented acute toxicity to humans and other organisms including birds and honey bees (Katagi & Tanaka, 2016; USEPA, 2008a). PHI- Pre-harvest Interval.

2.6 Diazinon

Diazinon, (*O, O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) (Figure 2.6.1), was first registered in the United States in 1956 and is sold under a variety of brand names as a pure substance, or mixed with other products (USEPA, 1988).

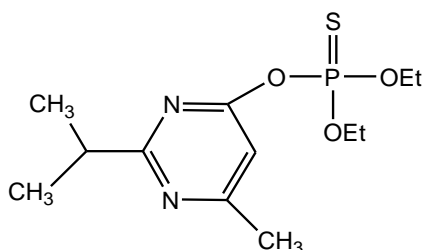


Figure 2.6.1: Molecular structure of diazinon. Adapted from Aggarwal et al. (2013)

2.6.1 Properties of diazinon.

The physico-chemical environmental fate properties of diazinon (Table 2.6.1) vary with environmental conditions, especially temperature (Pérez *et al.*, 2013). The octanol – air partition co-efficient (Log K_{oa}) value strongly predicts that air breathing organisms exposed to air contaminated with diazinon are likely to bioaccumulate the pesticide residues (Kelly *et al.*, 2007). The octanol-water partition coefficient (Log K_{ow}) value predicts minimal chances of bioaccumulation of diazinon residues in aquatic ecosystems (Kelly *et al.*, 2007). Henry's Law coefficients, vapor pressure, hydrolysis and photolysis half-lives indicate that diazinon readily partitions to organic matter, readily vaporizes, and can persist in the environment for long before complete dissipation (Barret & Jaward, 2012). Since environmental conditions keep changing, frequent review of diazinon persistence in vegetables should be done to check on pesticide safety levels. The dietary intake (ADI, ARfD, MRL and CT_{50}) and ecotoxicology (LC_{50} , LD_{50}) limits for various organisms in varied environmental matrices suggest that regular residue level monitoring should be done to minimize effects of contamination on the organisms.

Table 2.6.1: Physico-chemical environmental fate and ecotoxicological properties of diazinon

Property	Value at 20°C
Vapor pressure (mmHg)	9.10 X 10 ⁻⁵
Insecticide resistance classification	1B
Dissociation constant (pKa at 25°C)	2.6
Henry's Law constant	1.17 X 10 ⁻⁷
Maximum Residue Limits (mg/Kg)	0.05
Octanol - water partition coefficient (Log K _{ow})	3.81
Organic carbon-water partition coefficient (Log K _{oc})	1.602 – 2.635
Octanol – air partition co-efficient (Log K _{OA})	9.1
Hydrlyolysis half-lives in leafy plants (days)	0.6 -26
Solubility in water	40 mg/Litre
Dissipation rate RL ₅₀ on plant matrix	2.4 (range of 0.8 – 5.2) days
Bioconcentration factor (litres/Kg) CT ₅₀ 2 days	500
Ecotoxicology: Mammals acute oral LD ₅₀ (mg/Kg)	1139
Mammals dermal LD ₅₀ (mg/Kg b.w)	2000
Mammals inhalation LC ₅₀ (mg/l)	>5.0
Human- ADI (mg/Kg b.w day ⁻¹)	0.0002
Human- ARfD (mg/Kg b.w day ⁻¹)	0.025
Birds LD ₅₀ (mg/Kg)	1.44
Fish LC ₅₀ (mg/l)	3.1
Honey bees contact LD ₅₀ (mg/Kg)	0.13
Honey bees oral LD ₅₀ (mg/Kg)	0.09
Other pollinators LD ₅₀ (mg/Kg)	0.007 – 0.12

Adapted from Lewis *et al.*, (2016); USEPA (2006, 2008a)

2.6.2 Diazinon mode of action

Diazinon, a non-systemic organophosphate broadspectrum insecticide acts on target pests and some non-target organisms by inhibiting the enzyme acetylcholinesterase (AChE) (Francisco, 2011; Sharbidre *et al.*, 2011; Tova & Sherine, 2011). AChE hydrolyses the neurotransmitter acetylcholine (ACh) in animal cholinergic synapses and associated neuromuscular junctions (Francisco, 2011; van den Brink & Mann, 2011). The inhibition causes excessive accumulation of ACh in the animal nervous system blocking it from performing its physiological functions (Kuca *et al.*, 2006). The intoxicated animal dies due to overstimulation of cholinergic nervous

system. Consequently, animals including insects, birds, amphibians and mammals are poisoned (Francisco, 2011; Tova & Sherine, 2011). Diazinon on its own does not cause inhibition unless it is converted to the oxon transformation products including diazoxon and hydroxydiazoxon, *in vivo* (Coats, 1991; Wahla *et al.*, 1976). Animals with virtually no hydrolytic activity such as insects and birds are more sensitive to diazinon (Katagi & Tanaka, 2016; Mangas *et al.*, 2016; Michaela & Stanescu, 2014). Based on the cholinergic effects, its presence in the environment as residues in vegetables or in the air, may lead to devastating consequences in the natural ecosystems with disruptions in the food chains and extinction of some species (Elgueta *et al.*, 2017).

2.6.3 Effects of diazinon on human health.

While diazinon is registered as a general use pesticide in many countries including Kenya, chronic and acute dietary exposures of humans to diazinon have been associated with multiple health impacts including reproductive and developmental toxicity, neurotoxicity (Hancock *et al.*, 2008). The acute effects include headaches, stomachaches, vomiting, skin rashes, respiratory challenges, eye irritations, sneezing, seizures and coma (Antle & Pingali, 1994). Chronic health effects include reproductive dysfunctions, neuro behavioral disorders and birth defects (Kishi, 2005). Consequently, human dietary intake of diazinon is controlled by defining the acceptable quantities of the residues that can be taken per person per day and in a lifetime. The definitions are specified in the acute reference doses (ARfD), acceptable daily intake (ADI) from which health risk indices are computed (Darko & Akoto, 2008; FAO & WHO, 2010; Wang *et al.*, 2005). The ecotoxicology limits for diazinon in living organisms including man (Table 2.6.1) guided the Environmental Protection Agency of the United States of America action to limit indoor, lawn and home garden use of diazinon. Consequently, USEPA has been phasing it out from general use due to its toxicity and ecological risks to birds, fish and water invertebrates, and honey bees (USEPA, 2004). Due to these ecotoxicological effects,

diazinon has equally been banned in United Kingdom, Palestine and Mozambique (Pesticide Action Network-International, 2019b) due to health impacts on humans and other organisms in the environment. However, its use in production of *Brassica oleracea var. acephala* has not been evaluated for residue levels, consequently, the health risk levels of the pesticide and the resultant effects remains unclear.

2.6.4 Dissipation of diazinon in vegetables.

Similar to other pesticides used in crop production, dissipation and consequent persistence of diazinon on the target plants influence residual concentrations and subsequent exposure to the environment and consumers of the crops (Fantke *et al.*, 2012, 2013; Juraske *et al.*, 2008). Studies on dissipation half-lives for the recommended minimum and maximum rates of application of diazinon in different vegetables and study stations (Table 2.6.3) reported a range between 0.4 and 9.6 days, all within the RL₅₀ values acceptable for plant matrices (Lewis *et al* 2016). From Table 2.6.3, there is no variability in the dissipation half-life of diazinon with minimum and maximum rate of application in *Medicago sativa* (Talebi, 2006), *Poa annua* L. (Sears & Chapman, 1979), *Cichorium endivia* (Willis & McDowell, 1987), *Brassica oleracea var. Sabellica* (Willis & McDowell, 1987), *Olea europaea cv. Yacouti* (Cabras *et al.*, 1997), *Prunus persica* (Minelli *et al.*, 1996), *Allium fistulosum* L. (Ettiene *et al.*, 2006), *Lycopersicon esculentum* Mill. (Antonious, 2005), *Lycopersicon esculentum* Mill. *cv. Rio Grande* (Prieto *et al.*, 2002), *Lycopersicon esculentum* Mill. (Lindquist & Krueger, 1975) and *Triticum aestivum* L. (Willis & McDowell, 1987). However, significant variability in dissipation half-lives were reported in *Brassica oleracea alboglabra cv. Guy Lon* (Ripley *et al*, 2003), *Brassica campestris* (Khay *et al.*, 2006; Willis & McDowell, 1987), *Poa pratensis* L. (Sears *et al.*, 1987), *Poa pratensis* L. (Kuhr & Tashiro, 1978), *Gossypium hirsutum* L. (Willis & McDowell, 1987), *Zea mays* L. (Willis & McDowell, 1987) and Turfgrass (Lemmon & Pylypiw, 1992). Dissipation in the leaves has the lowest RL₅₀ values, ranging between 0.4 and 5.3 days. Fruity plants have

higher RL₅₀ than leafy plants ranging between 0.8 and 9.6 days. While variations in RL₅₀ may be associated to phenological and morphological differences across sets of vegetables, it is not clear how characteristics of *Brassica oleracea* var. *acephala* grown at KOSFIP may influence RL₅₀ values.

Table 2.6.3: Summary of dissipation half-lives for minimum and maximum application rates of diazinon in different vegetables and study stations.

Vegetable	Botanical name	Study station	Plant matrix	Half-life (RL ₅₀) Ranges			References
				Min	Max	Mean	
Alfalfa	<i>Medicago sativa</i>	Iran	Leaves	1.8	1.8	1.8	(Talebi, 2006)
Annual bluegrass	<i>Poa annua</i> L.	Canada	Turf	2.6	2.6	2.6	(Sears & Chapman, 1979)
Chinese broccoli	<i>Brassica oleracea alboglabra</i> cv. <i>Guy Lon</i>	Canada	Leaves	2	2.5	2.25	(Ripley et al., 2003)
Chinese cabbage	<i>Brassica campestris</i>	Republic of Korea	Leaves	1.3	1.5	1.4	(Khay et al., 2006)
Cotton	<i>Gossypium hirsutum</i> L.	AZ, USA	Leaves	1.2	2.5	1.85	(Willis & McDowell, 1987)
Endive	<i>Cichorium endivia</i>	Canada	Leaves	2.2	2.2	2.2	(Willis & McDowell, 1987)
Kale	<i>Brassica oleracea</i> var. <i>Sabellica</i>	DC, USA	Leaves	5.3	5.3	5.3	(Willis & McDowell, 1987)
Kentucky bluegrass	<i>Poa pratensis</i> L.	Canada	Blades	1.1	5.4	3.25	(Sears et al., 1987)
Kentucky bluegrass	<i>Poa pratensis</i> L.	NY, USA	Blades	5.3	6.9	6.1	(Kuhr & Tashiro, 1978)
Maize	<i>Zea mays</i> L.	IA, USA	Leaves	0.4	1.8	1.1	(Willis & McDowell, 1987)
Norway maple	<i>Acer platanoides</i>	MS, USA	Leaf litter	2.13	8.25	5.19	(Moore et al., 2007)
Olive	<i>Olea europaea</i> cv. <i>Yacouti</i>	Italy	Fruit	9.6	9.6	9.6	(Cabras et al., 1997)
Peach	<i>Prunus persica</i>	Brazil	Fruit	5.2	5.2	5.2	(Minelli et al., 1996)
Spring onion	<i>Allium fistulosum</i> L.	Venezuela	Leaves	1.03	1.03	1.03	(Ettiene et al., 2006)
Tomato	<i>Lycopersicon esculentum</i> Mill.	-	Leaves	0.8	0.8	0.8	(Antonious, 2005)
Tomato	<i>Lycopersicon esculentum</i> Mill. cv. <i>Rio Grande</i>	Venezuela	Fruit	1.14	1.14	1.14	(Prieto et al., 2002)
Tomato	<i>Lycopersicon esculentum</i> Mill.	OH, USA	Fruit	0.8	0.8	0.8	(Lindquist & Krueger, 1975)
Turfgrass	-	USA	Blades	2.1	4.1	3.1	(Lemmon & Pylypiw, 1992)
Wheat	<i>Triticum aestivum</i> L.	TX, USA	Leaves	5	5	5	(Willis & McDowell, 1987)

Notes: Diazinon dissipation half-lives in various vegetable matrices indicate variability with vegetable type and site of study.

2.6.5 Diazinon dissipation processes and resultant transformation products.

Dissipation processes that directly contribute to reduction of contact diazinon concentration on vegetables include photodecomposition, volatilization, diffusion, wash-off, growth dilution and chemical degradation (Coats, 1991; Fantke & Juraske, 2013). The processes are influenced by environmental factors such as temperature, light intensity, humidity, precipitation, and wind speed (Edwards, n.d.; Lewis & Tzilivakis, 2017; Mahmood et al., 2016). Plant species characteristics and physico-chemical properties of pesticide formulation, stability, bioavailability and efficacy also contribute to dissipation of pesticides from plant surfaces (Australian Pesticides and Veterinary Medicines Authority, 2003; Farha et al., 2016). While photodecomposition, volatilization and wash-off processes may be changing with weather and leaf characteristics (Delcour *et al.*, 2015; Nurhayati, 2011), hydrolysis remains the principal chemical dissipation process following foliar application of aqueous diazinon (Freed *et al.*, 1979; Kouloumbos *et al.*, 2003; Ku & Chang, 1998; Lacorte *et al.*, 1995; Pineda, 2001). The general hydrolysis scheme is shown in Figure 2.6.2.

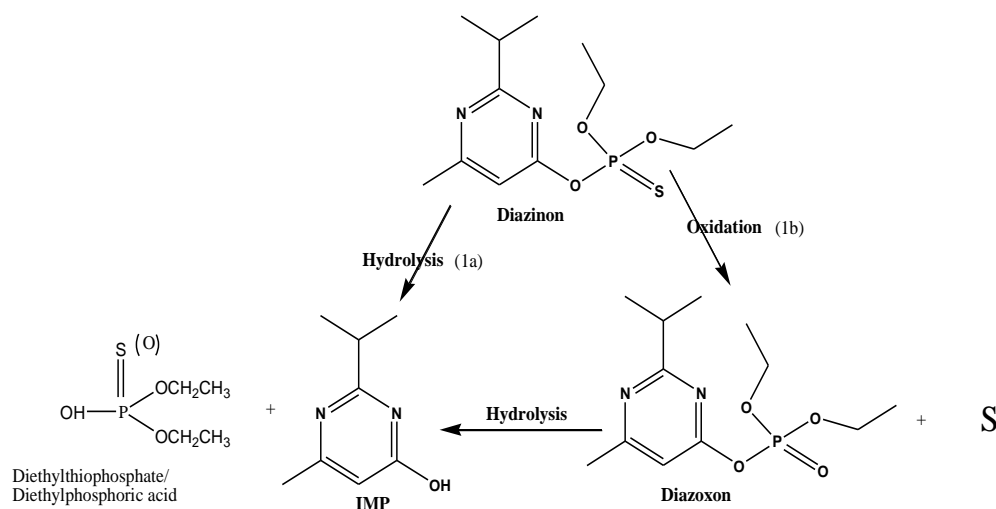


Figure 2.6.2: General scheme for hydrolysis of diazinon: direct hydrolysis and oxidative desulfuration.

Notes: S is sulphur atom resulting from desulfuration of diazinon to form diazoxon. Adapted from APVMA (2003); Kouloumbos *et al.* (2003).

In the general hydrolysis scheme (Figure 2.6.2), pathway **1a** is the direct hydrolysis of diazinon with the formation of 2-isopropyl-6-methyl-4-hydroxypyrimidin-4-ol (IMP) and diethylthiophosphate (Kouloumbos *et al.*, 2003). The OH radical resulting from UV irradiation (photolysis) of aqueous diazinon attacks the diazinon molecule at the pyrimidine - ester bond causing cleavage of the molecule (Figure 2.6.3) (Kouloumbos *et al.*, 2003; Shemer & Linden, 2006).

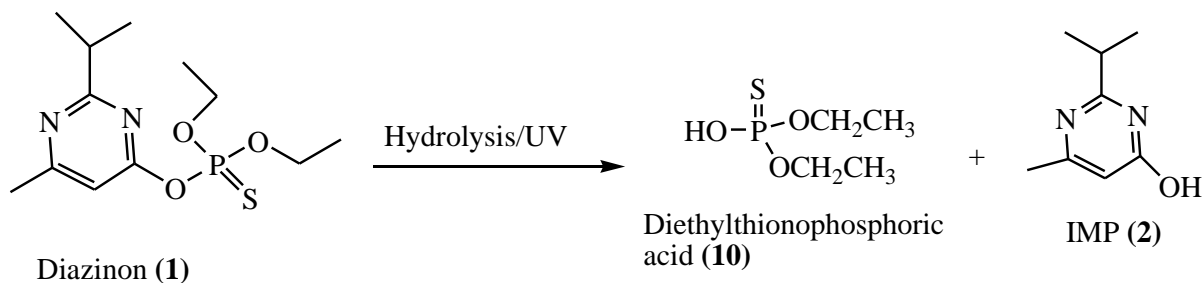


Figure 2.6.3: Photolysis aided hydrolysis leading to cleavage of diazinon.

Pathway **1b** (Figure 2.6.2) is an oxidative desulfuration process and begins with the oxidation

of diazinon to form diazoxon and a sulphur atom (Australian Pesticides and Veterinary Medicines Authority, 2003; Kouloumbos *et al.*, 2003). The process is similarly aided by photolysis catalyzed OH radical attack on the thiono (P=S) group on diazinon. The diazoxon formed readily hydrolyses to form IMP and either diethylthionophosphoric acid or diethylphosphoric acid (Figure 2.6.4 and Figure 2.6.5).

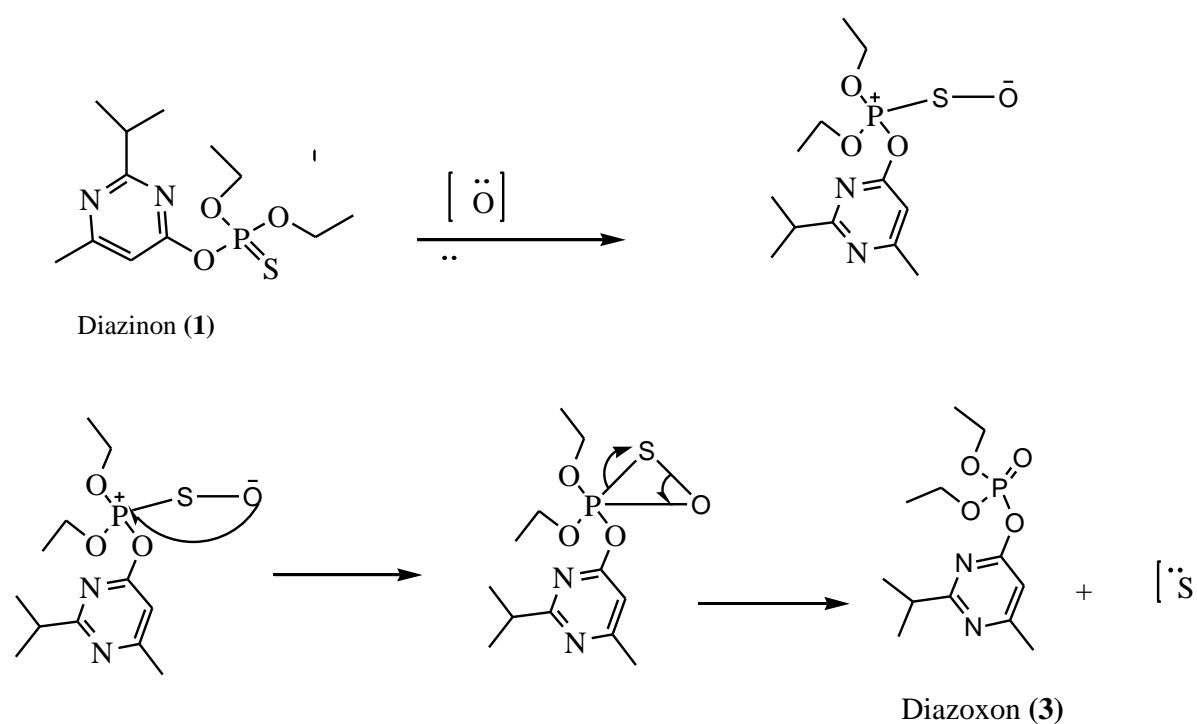


Figure 2.6.4: Mechanism for the oxidation of diazinon (1) to form diazoxon (3) and elemental sulphur (S).

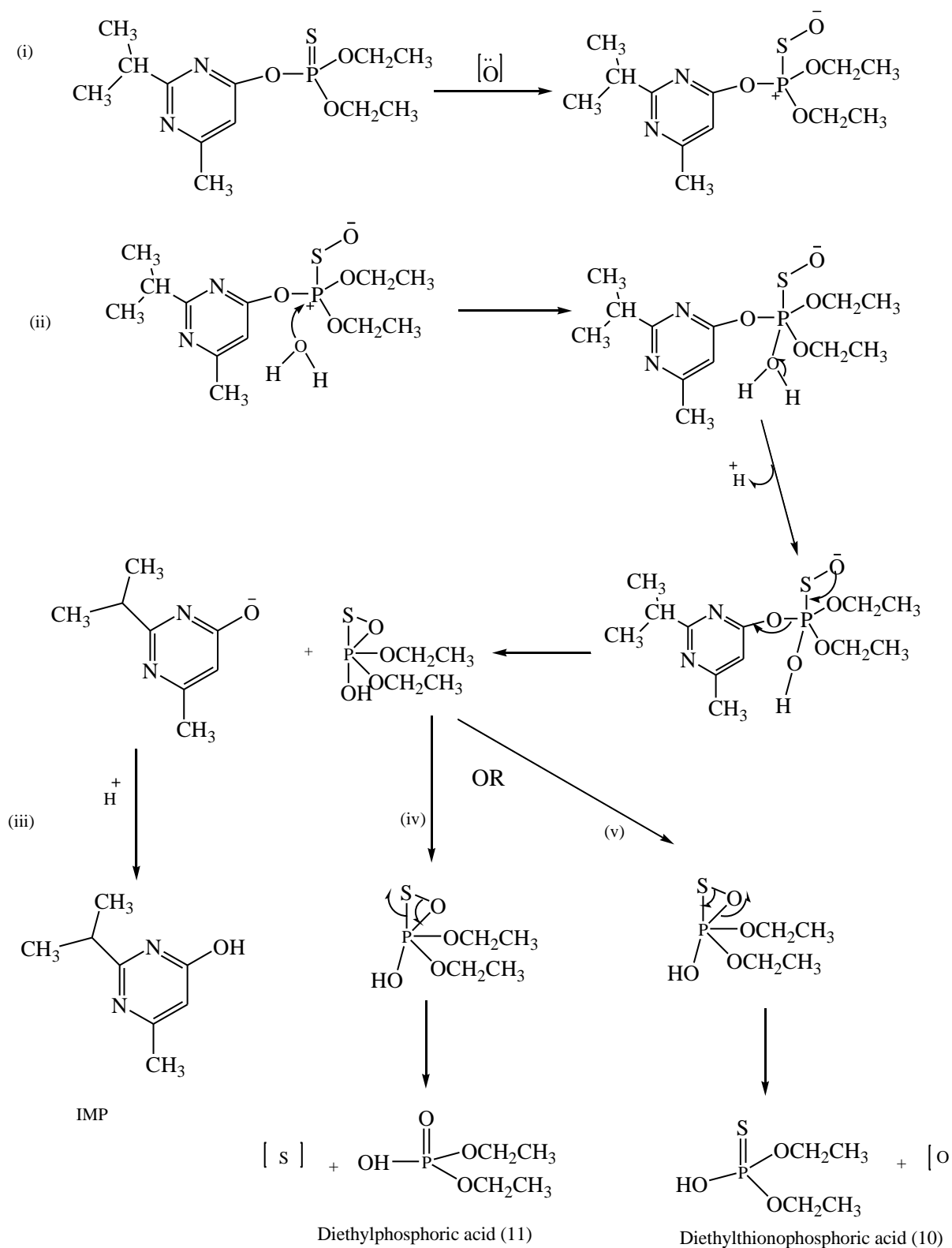


Figure 2.6.5: Mechanism for the oxidation of diazinon (1) and subsequent hydrolysis of diazoxon (3) to form IMP (2), diethylthionophosphoric acid (10) and diethylphosphoric acid (11).

Experiments under trace amounts of water with either inadequately stabilized liquid

formulations of diazinon stored at elevated temperatures, or in corroded metal containers or transparent bottles, have shown that diethylphosphoric acid (**11**) and diethylthionophosphoric acid (**10**) molecules formed through pathways **1a** and **1b** may undergo further reactions (Figure 2.6.6) (Australian Pesticides and Veterinary Medicines Authority, 2003; Freed *et al.*, 1979). The toxic products, *O,O,O',O'*-tetraethyl dithiopyrophosphate (*S, S* – TEPP or sulfotepp) (**13**) and *O,O,O',O'*- tetraethyl-monothiopyrophosphate (*O, S* –TEPP or monot Tepp) (**12**), respectively, may be better pesticides than diazinon (Australian Pesticides and Veterinary Medicines Authority, 2003; Freed *et al.*, 1979; Manal *et al.*, 2008).

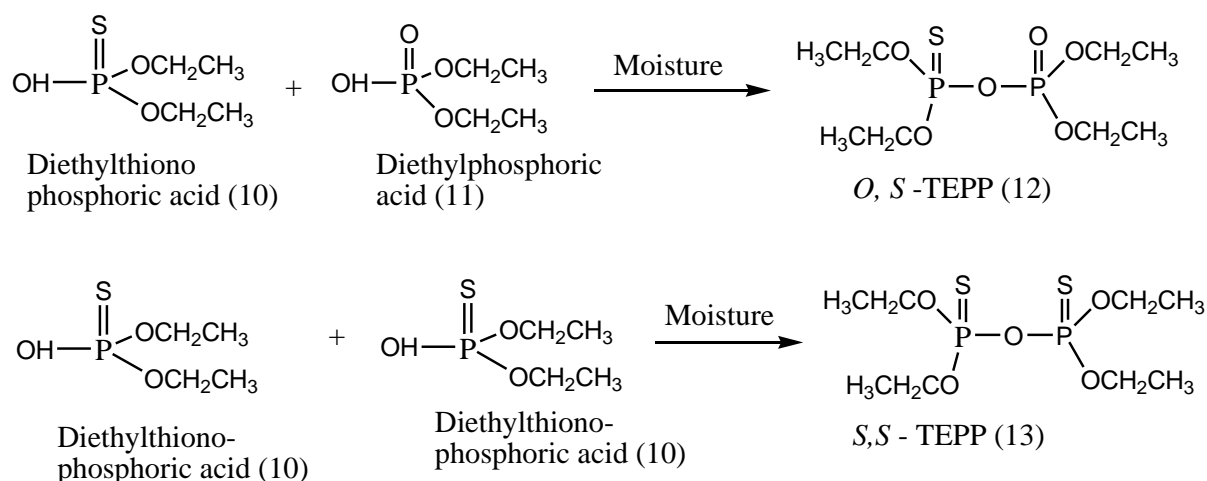


Figure 2.6.6: Formation of transformation products, sulfotepp and monot Tepp.

Notes: *S, S* – TEPP: *O,O,O',O'*-tetraethyl dithiopyrophosphate (sulfotepp); *O, S* –TEPP: *O,O,O',O'*- tetraethyl-monothiopyrophosphate (monot Tepp). Adapted from APVMA (2003).

The third and alternative pathway to direct hydrolysis retains the thiophosphonic moiety of diazinon (**1**) but targets the primary and secondary carbon atoms of the isopropyl chain for hydroxylation. The hydroxylation of the primary carbon yields hydroxydiazinon (**4**) followed with oxon metabolite hydroxydiazoxon (**6**) and diazinon aldehyde (**9**) as products of oxidative

desulfuration and further oxidation, respectively (Figure 2.6.7).

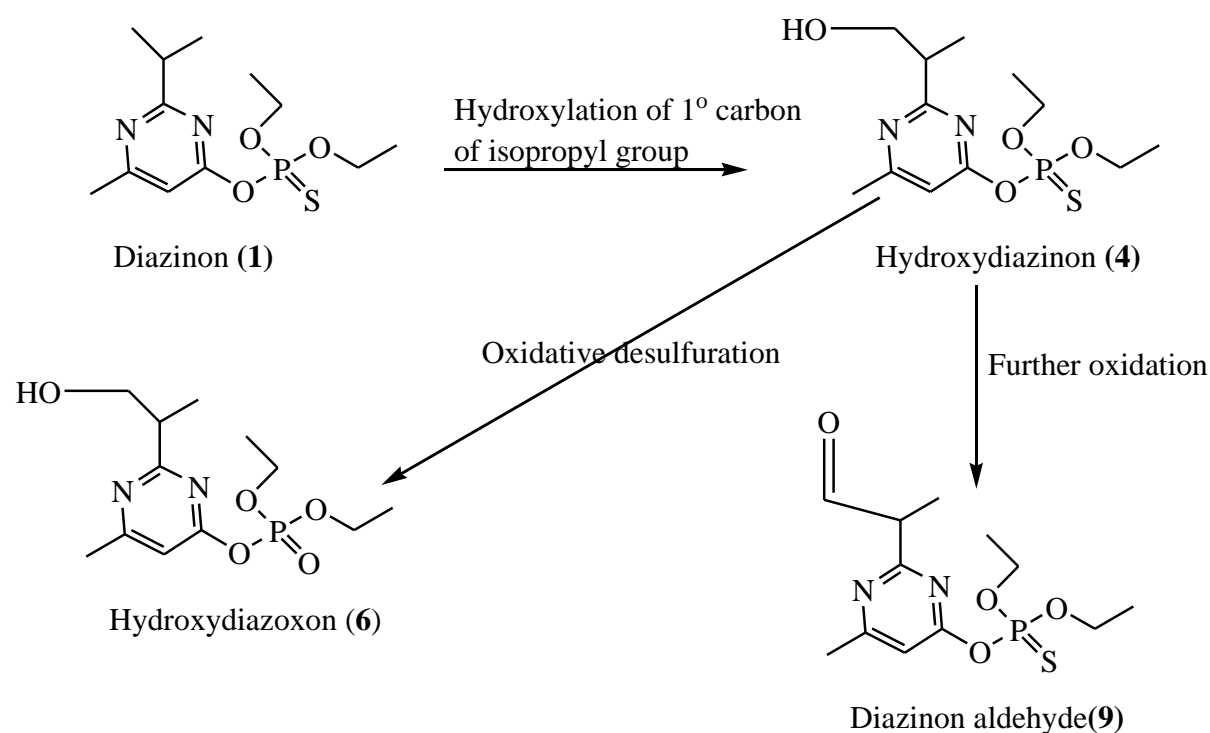


Figure 2.6.7: Transformation products resulting from hydroxylation of primary carbon of the pyrimidine group of diazinon molecule.

When the secondary carbon of the isopropyl chain is targeted for hydroxylation, similar to primary carbon, transformation product 2-hydroxydiazinon (5) is formed (Figure 2.6.8) (Arief *et al.*, 2015; Kouloumbos *et al.*, 2003). Subsequent oxidative desulfuration and further oxidation yields 2-hydroxydiazoxon (7) and diazinon methyl ketone (8), respectively.

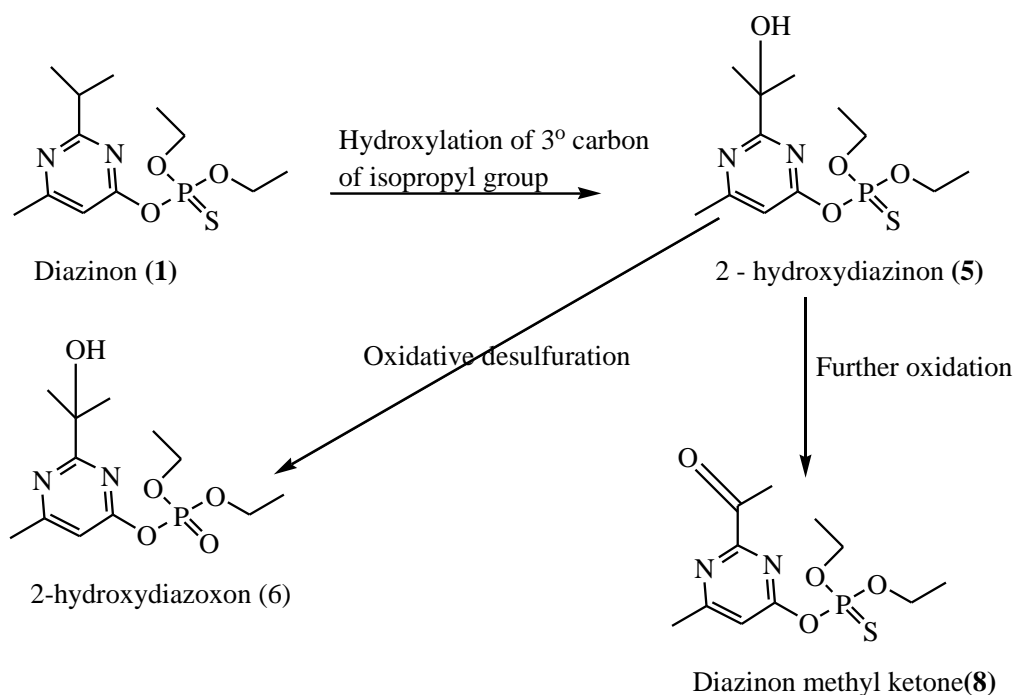
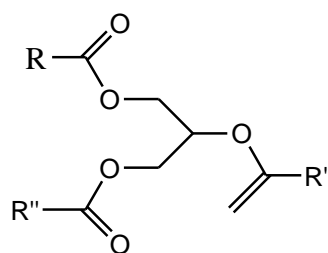


Figure 2.6.8: Transformation products resulting from hydroxylation of tertiary carbon of the pyrimidine group of diazinon molecule.

Upto 1984, the occurrences of toxic sulfotepp transformation products of diazinon in crops at harvest or in processed foods were recurrent but have been minimized (Australian Pesticides and Veterinary Medicines Authority, 2003; EFSA, 2010). Currently manufactured liquid formulations of diazinon which are based on hydrocarbon solvents are stabilized with epoxidized soybean oil (Figure 2.6.9) to deter formation of *O,O,O',O'*-tetraethyl dithiopyrophosphate (*S,S* – TEPP or sulfotepp) (13) and *O,O,O',O'*- tetraethyl-monothiopyrophosphate (*O,S* – TEPP or monotep) (12) (Australian Pesticides and Veterinary Medicines Authority, 2003).



Where R, R' and R'' are epoxidized hydrocarbon chains given by

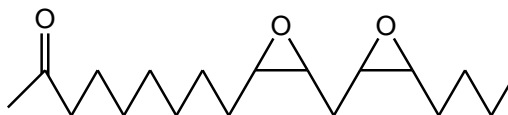
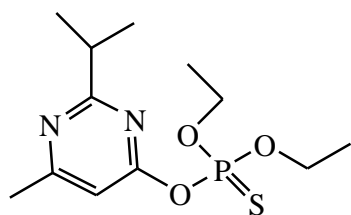
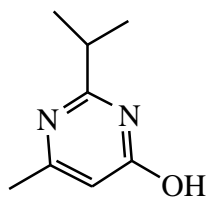


Figure 2.6.9: Molecular structure of epoxidized soybean oil molecule. Adapted from EFSA (2004).

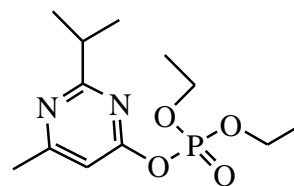
Subsequently, the toxic sulfotepp transformation products have not been evaluated as residues of diazinon and listed with maximum residue limits and corresponding dietary intake definitions to guide subsequent health risk determinations. Consequently, their levels in *Brassica oleracea var. acephala* of KOSFIP have not been considered in this study. Overall, some of the metabolites and transformation products which may be resulting from a combination of factors that influence physical and chemical dissipation of diazinon on plants, in animals that get exposed to residues and in the environment (Coats, 1991) are listed in Figure 2.6.10.



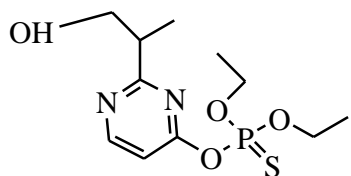
Diazinon(1)



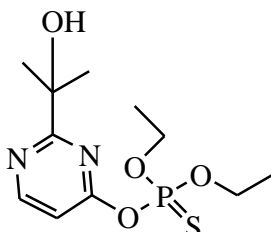
IMP (2)



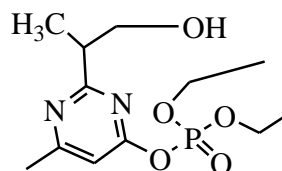
Diazoxon (3)



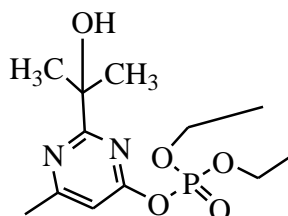
Hydroxydiazinon(4)



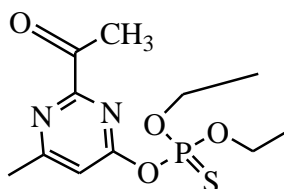
2-hydroxydiazinon (5)



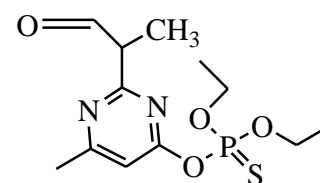
Hydroxydiazoxon (6)



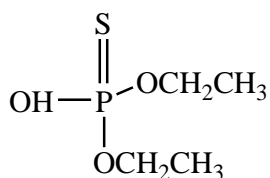
2-hydroxydiazoxon (7)



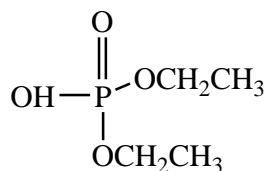
Diazinon methyl ketone (8)



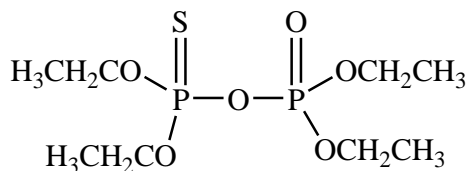
Diazinon aldehyde (9)



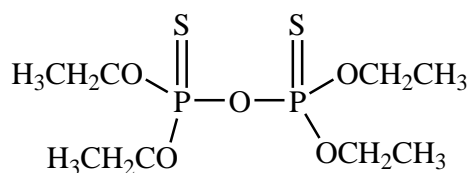
Diethylthionophosphate (10)



Diethylphosphoric acid (11)



O,S - TEPP (12)



S,S - TEPP (13)

Figure 2.6.10: Structures of transformation products (1 – 13) that may be formed during chemical dissipation of diazinon.

2.6.6 Fate of diazinon chemical dissipation transformation products.

Green leafy plants treated with diazinon recognize transformation products and metabolites as xenobiotics (Ramel *et al.*, 2012) which may readily be biotransformed in the plant tissues through either conversion, conjugation or compartmentation by the plant cytochrome P450 monooxygenases and glutathiones (Coats, 1991; Dębski *et al.*, 2007; Gorinova *et al.*, 1999; Poet *et al.*, 2003). These biotransformation processes aid dissipation and subsequent decline in residual quantities of the pesticide with time post application. The efficiency of biotransformation varies with phenological and physiological stages besides plant species characteristics (Gorinova *et al.*, 1999; Jacobsen *et al.*, 2015). Studies on diazinon stability and dissipation in varied environmental matrices (water, soil, fruits and vegetables) with different dosages reported that approximately 90% of initial amount of diazinon was dissipated, with about 40 % being IMP (Bavcon *et al.*, 2003; Elersek & Filipic, 2011; Zabar, 2012). The difference in quantities reported could be diazinon residues detectable by chemical analysis methods. Consequently, diazinon residues on the leaves of green vegetables may be the only detectable toxins.

2.6.7 Recommended application conditions of diazinon in *Brassica oleracea var. acephala* production in Kenya.

Diazinon is registered by PCPB for use in brassica vegetables against varied pests (Pest Control Products Board, 2018) and is one of the popular pesticides used by smallholder farmers in Kenya (Nyakundi *et al.*, 2010). Diazinon is available to farmers in various formulations from different manufacturers (Pest Control Products Board, 2018). The registration recommends application conditions of about 1.25 litres per hectare, re-entry period of 24 hours, pre-harvest interval of 12 days (Appendix H_A; Appendix H_B) to attain maximum residue levels ≤ 0.05 mg/Kg in the brassicas (Barret & Jaward, 2012; FAO, 2010b; Pest Control Products Board, 2018). Despite availability of data from many parts of the world (Lewis *et al.*, 2016; Lewis &

Tzilivakis, 2017), data on field trials with various vegetables from across Kenya are not available. Consequently, the recommended application conditions may have been extrapolated from other regions of the world. It is therefore not determined if the recommended application conditions of rates and pre-harvest intervals are suitable to the local environment in Kenya and the area of study.

2.6.8 Analysis of diazinon residues in vegetables

Chromatographic technology instruments such as GC – MS/MS and LC-MS/MS with appropriate detectors are preferred for the detection and quantification of pesticide residues in vegetables (Kouloumbos *et al.*, 2003; Raina, 2011; Raina & Hall, 2008; Tova & Sherine, 2011). The instruments guarantee wide range of sensitivities, separation power, selectivity and identification capabilities, and ability to screen multiple pesticides and their transformation products from wide variety of chemical classes in very complex matrixes in a single run. Negative chemical ionization (NCI) and electron spray ionization (ESI) are considered most appropriate detectors (Riana & Hall, 2009). However, NCI has LOQs and LODs of 0.02 mg/Kg while ESI has LOQs and LODs of 0.01 mg/Kg. The very low and equal LOQs and LODs make ESI a most appropriate detector for the hazardous diazinon molecules. Samples prepared by QuEChERS- based multiple residue methods with LC-ESI-MS/MS have reported between two and 150 pesticides (Afify *et al.*, 2010; Andrade *et al.*, 2015; Hanafi *et al.*, 2018). This method suits diazinon residues due to its toxicity low maximum residue limits in vegetables formation of very complex samples with residues at trace levels.

2.7 Monitoring levels of pesticide residues in farm-gate baskets

Monitoring programs and evaluation studies at diverse geographical locations report presence of pesticide residues in vegetables (EFSA, 2010; European Commission, 2019). Globally, food safety standards of vegetables, for example *Brassica oleracea var. acephala*, are judged based

on Codex, regional and national MRLs (Figure 2.7.1) (FAO & WHO, 2010). While most countries adopt the Codex MRLs, regional and national legislations may develop action MRL standards for purposes of international trade (WHO & FAO, 2019). The European Union (EU) MRL value of 0.01 mg/Kg is the default MRL for *Brassica oleracea var. acephala* because the use of diazinon has been banned in the EU markets. Australia and Kenya have adopted the Codex MRL of 0.05 mg/Kg (Australian Pesticides and Veterinary Medicines Authority, 2003; Pest Control Products Board, 2018) for locally consumed produce and to international markets except for EU that observe action MRL of 0.01 mg/Kg (Pesticide Action Network-International, 2019b).

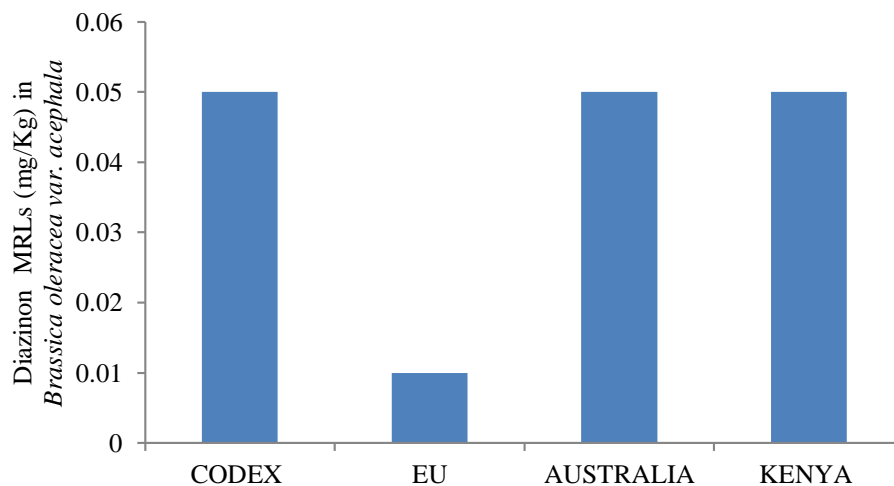


Figure 2.7.1: Acceptable maximum residue limits (MRL) for diazinon in *Brassica oleracea var. acephala* for Codex, European Union, Australia and Kenya.

Pesticide residue screenings at the farm-gates have received much attention as a strategy for monitoring of potential health hazards that may affect vegetable food safety (Jallow, Awadh, Albaho, Devi, & Ahmad, 2017; Xu *et al.*, 2018). The strategy is a response to the high levels of pesticide residue contamination reported in foods even when good agricultural practices (GAP) are observed (Akan, Battah, *et al.*, 2015; Akan, Musa, *et al.*, 2015; M. Musa *et al.*, 2015; Mutai *et al.*, 2015). To mitigate the health risk effects due to consumption of pesticide residue

laden vegetables and the environmental pollution due to pesticide use, developed nations such as Denmark, the Netherlands, Norway and Sweden have adopted strategies to reduce use of synthetic pesticides (Hoi *et al.*, 2016). The nations have targeted withdrawal of over 60% of pesticide active ingredients (Directive 91/414/EEC in 2010) and continuous review of MRL legislation directives. The directives were responses to recommendations following farm-gate surveys of their vegetables. Some few developing countries such as Nicaragua, Indonesia, Turkey and China made efforts, setting targets to reduce pesticide use (Bakircı *et al.*, 2014; Hoi *et al.*, 2016; Xu *et al.*, 2018). Many developing countries, including Kenya, have not established consistent farm-gate monitoring strategies for locally produced and consumed vegetables (FAO, 2007c; Hoi *et al.*, 2016; Jallow, Awadh, Albaho, Devi, & Thomas, 2017a; Mutai *et al.*, 2015; Mwanja *et al.*, 2017; Njoku *et al.*, 2017; Nthiga, 2012). Consequently, farm-gate pesticide residue burden remains undetermined and there are no bases for strategic actions including reduction of pesticide use in agricultural production.

Studies with various vegetables across the globe report levels of diazinon and respective health risk indices (Table 2.7.1).

Vegetables grown in Thailand (Sapbamrer & Hongsibsong, 2014; Wanwimolruk *et al.*, 2015), Spain (Fenoll *et al.*, 2007) and Iran (Rohani *et al.*, 2017) report diazinon residual levels below Codex MRL with corresponding health risk indices below 1.0. Vegetables with these food safety parameters pose no immediate health risks to the consumers. Such safe conventional produce may be associated with farmers skilled in pesticide use thus observing good agricultural practices (GAPs). It may also be concluded that in the regions of such produce, government legislation and surveillance policies are adequately observed to ensure sustainable vegetable farming. On the contrary, vegetables treated with diazinon in Bangladesh (Prodhan *et al.*, 2018; Rohani *et al.*, 2017; Sultan *et al.*, 2016), Pakistan (Latif *et al.*, 2011), Nigeria (Akan, Jafiya, *et al.*, 2013), Ghana (Bempah *et al.*, 2012) and Sudan (Awad *et al.*, 2018)

reported wide variations in diazinon residual levels with most vegetables reporting above Codex MRLs and HRI greater than 1.0 (Table 2.7.1). The vegetables therefore pose health risks to the consumers. The residual levels suggest that the use of pesticides may be unregulated, and legislations and surveillance policies on use of pesticides may be inadequate.

Table 2.7.1: Diazinon residue levels and HRI in various farm-gate vegetables from different parts of the world.

Country	Vegetable	Conc. (mg/Kg)			References
		and MRLs	EDI	HRI	
Bangladesh	Egg plant	2.4835(0.01)	0.014	17.0	(Alam et al., 2015)
	Egg plant	0.059(0.2)	0.000	0.0	(Prodhan et al., 2018)
	Tomatoes	2.75(0.01)	0.016	14.0	(Alam et al., 2015)
	Cauliflower	0.402(0.01)	0.002	0.8	(Sultan et al., 2016)
	Yard long bean	0.0195(0.01)	0.000	0.0	(Sultan et al., 2016)
	Yard long bean	0.060(0.2)	0.000	0.0	(Prodhan et al., 2018)
Spain	Tomatoes	0.3612(0.01)	0.002	0.7	(Fenoll et al., 2007)
Pakistan	Apple	0.038(0.001)	0.000	0.0	(Asi, 2003)
	Banana	1.6(0.001)	0.011	11	(Latif et al., 2011)
Iran	Tomatoes	0.276(0.05)	0.002	0.8	(Rohani et al., 2017)
Thailand	Chinese kale	0.012(0.01)	0.000	0.0	(Sapbamrer & Hongsibsong, 2014)
	Chinese kale	0.01475(0.05)	0.000	0.0	(Wanwimolruk et al., 2015)
	Spring onion	0.245 (0.02)	0.001	0.5	(Sapbamrer & Hongsibsong, 2014)
	Parsley onion	0.009(0.05)	0.000	0.0	(Sapbamrer & Hongsibsong, 2014)
	Ginger	0.012(0.05)	0.000	0.0	(Sapbamrer & Hongsibsong, 2014)
Nigeria	<i>T. occidentalis</i>	0.101(0.02)	0.001	0.2	(Njoku et al., 2017)
	<i>C. argentea</i>	0.102(0.02)	0.001	0.2	(Njoku et al., 2017)
	Watermelon	0.495(0.04)	0.003	1.0	(M. Musa et al., 2015)
	Spinach	97.745(0.04)	0.562	187.0	(Akan et al., 2013)
	Onions	20.00(0.02)	0.115	38.0	(Akan et al., 2013)
Ghana	Tomatoes	3.612 (0.01)	0.021	7.0	(Bempah et al., 2012)
Sudan	Cucumber	25.86 (0.1)	0.150	50.0	(Awad et al., 2018)
	Tomatoes	2.55 (0.1)	0.015	5.0	(Awad et al., 2018)
Kenya	<i>Brassica oleracea</i> <i>var acephala</i>	0.18(0.01)	0.001	0.4	(Karanja et al., 2012)
	<i>Brassica oleracea</i> <i>var acephala</i>	0.00168/ 0.0019 (w/d)(0.05)	0.00/ 0.00	0.00/ 0.00	(Ngolo et al., 2019)
	Tomatoes	0.0013/0.003 (w/d)(5)	0.00/ 0.00	0.00/ 0.00	(Ngolo et al., 2019)

MRL- Maximum Residue Limits; EDI- Estimated Daily Intake; HRI- Health Risk Index; HRI > 1.0 is a health risk to consumers; w/d – wet and dry seasons.

Most pesticide residue studies in Kenya have targeted persistent organochlorines in soil (Getenga et al., 2004), sediments (Abong'o, 2009; Osoro et al., 2016; Nyaundi et al., 2019), surface water (Osoro et al., 2016), fish (Madadi, 2005) and marine water (Wandiga et al., 2002). Residue studies on organophosphates have been limited to chlorpyrifos and dimethoate (Mutai et al., 2016; Okworo, 2017) and in agricultural soils, river sediments, river and lake waters and aquatic animals (Abbasi & Mannaerts, 2018; Abong'o, 2009; S. Musa et al., 2011). There are also pesticide residue studies done on horticultural crops such as French beans, peas and flowers for export markets (Mwaniki, 2017). However, limited studies on residual levels of pesticides have been reported for food crops grown for local consumption. Some of the reported residual levels of diazinon in *Brassica oleracea var. acephala* were determined in Kenya and the findings show that the levels were below Codex MRLs with HRI <1.0 (Karanja et al., 2012; Ngolo et al., 2019). However, data on diazinon residues in locally grown and consumed vegetables such as *Brassica oleracea var. acephala* are limited. Given that regular vegetable safety surveillance through farm-gate screening has not been going on in Kenya, the pesticide contamination levels of vegetable produce and the resultant health risks to the consumers are undetermined.

In KOSFIP area, the use of synthetic pesticides in production of vegetables such as *Brassica oleracea var. acephala* is increasing with the increasing pest infestations (MoALF, 2016). Popular pesticides including broad spectrum diazinon is used by farmers (J, Onyango, Personal communication, June 20, 2018) who may be inadequately informed on the dangers associated with improper use of the pesticides. Consequently, it is not established how the levels of pesticides in the farm-gate baskets of *Brassica oleracea var. acephala* produced at KOSFIP compare with the FAO/WHO MRLs.

2.8 Influence of rate of application on pesticide residue levels in vegetables

Rate of application of a pesticide is the label instruction of the amount of the active ingredient or pesticide formulation expressed in units of volume or weight per unit area delivered in a pesticide application for a particular crop using a calibrated applicator equipment (Eldridge, 2008). As a recommendation of the manufacturer, rate of application provides the highest possible dose of the pesticide that will effectively control or kill the most tolerant target pest without causing unacceptable environmental contamination (Gressel, 2017). Field trials in Thailand, Finland and Germany with different rates of diazinon in kale and Chinese cabbage indicated that resultant residual levels varied with rate of application, vegetable species and site of application (Lewis & Tzilivakis, 2017). Similarly, trial on *Vitis vinifera* (L.) using recommended and double recommended application rates (Table 2.8.1) showed significant differences in dissipation half-lives and safe pre-harvest intervals at which the MRLs of carbendazim and mancozeb were realized (Banerjee, 2005).

Table 2.8.1: Effect of carbendazim and mancozeb application rates on dissipation RL_{50} and PHI on *Vitis vinifera* (L.)

Pesticide	Dosage	Dissipation half-lives RL_{50} (days)	Pre-Harvest Intervals (days)
Carbendazim	Recommended	7.3	26
	Double	4.6	33
Mancozeb	Recommended	8.1	12
	Double	5.7	17

Notes: Increased dosages correspondingly affected RL_{50} and safe PHIs for both pesticides. Adapted from Banerjee (2005).

While the recommended doses are based on outcomes of supervised trials (Lewis & Tzilivakis, 2017; MIT, 1991), the changing environmental conditions of temperature, humidity, solar radiation and air speed modify both vegetable and pest biological processes (Elad & Pertot, 2014; Nurhayati, 2011). The consequent changes in chemical interactions make the pesticide dissipation rates and residue concentrations difficult to predict (Cilas *et al.*, 2016). In addition, the rapidly changing climate, and the internal parameters such as physico-chemical properties

of the pesticide, and the morphology and phenology of the vegetable plant may also influence the dissipation rate of the pesticide (Cabras et al., 1988; Hanafi et al., 2018; Prodhan et al., 2018; Pujeri et al., 2016; USEPA, 2004).

Some field experiments with recommended rates of application have reported detectable levels of residues below the MRLs at PHI (Fantke & Juraske, 2013; Kiwango *et al.*, 2018) while other experiments reported levels higher than the MRLs at PHI even when GAPs were observed (Akan, Musa, *et al.*, 2015; Jallow, Awadh, Albaho, Devi, & Ahmad, 2017; M. Musa *et al.*, 2015; Mutai *et al.*, 2015). Even though residual levels of pesticides are dependent on rate of application (Horvath *et al.*, 2014; MacLachian & Hamilton, 2011; Sadlo, 2000), varying dissipation patterns have been reported for different pesticides in vegetables (Ramírez-Bustos *et al.*, 2019). Consequently, the reliability of the label prescribed rates of application in every environment may be doubtful.

In hot and humid regions with scanty rainfall where pest infestation is diverse and rapid, farmers often over-rely on pesticides for the management of pests and diseases (Cilas *et al.*, 2016; Elad & Pertot, 2014; Nnamonu & Onekutu, 2015). The farmers may disregard GAPs and resort to overdose by either using multiple pesticides on one crop or by using higher concentrations of a pesticide than what is prescribed on the labels. Such instances of disregard for dosages have been reported among smallholder farmers in parts of China (Yang, 2007), Vietnam (Hoi *et al.*, 2016), Kuwait (Jallow, Awadh, Albaho, Devi, & Thomas, 2017a), Zambia (Mwanja *et al.*, 2017), Tanzania (Kiwango *et al.*, 2018; Mahugija *et al.*, 2017; Nonga *et al.*, 2011) and Kenya (Nyakundi *et al.*, 2010) when farmers were faced with several pest complexes but without viable alternatives for managing the pest invasions. The reports have not shown whether the farmers were able to extend the PHI of the pesticides to match the high dosages, neither did the reports declare the effect of the dosages on the transformation products in the exceeded MRLs. The KOSFIP area of Homa Bay County of Kenya experiences similarly hot

and humid weather conditions with scanty rainfall throughout the year (Jaetzold *et al.*, 2009; MoALF, 2016). The smallholder farmers of *Brassica oleracea var. acephala* in the study area are faced with multiple pests and diseases against which they use broad spectrum diazinon to manage. While overdose of the pesticide may be a common practice of farmers similar to other regions, it is not documented how the rate of application of diazinon influences residue levels and PHI of diazinon in *Brassica oleracea var. acephala* grown within the KOSFIP area.

2.9 Effect of post application durations on pesticide residue levels in vegetables

The post application duration is the time between the last application of a pesticide on a crop and when it is harvested for consumption (Pujeri *et al.*, 2016; USEPA, 2008b). The period is observed to allow for optimum dissipation of the pesticide in the produce for food safety purposes (Alister *et al.*, 2017; Kiwango *et al.*, 2018). These periods are experimentally determined through pesticide trials (Table 2.9.1) and are specific for registered pesticide – vegetable combinations, giving a standard parameter of good agricultural practices (GAP) called pre-harvest interval (PHI) (PMRA, 2009). Table 2.9.1 displays the effect of post application periods on the determination of pre-harvest intervals. The initial concentrations were different for all the vegetables. This suggested that the rates of application and dissipation patterns were also different. However, as the post application periods increased, residual levels tended towards MRL values and below. Instances where the label PHI was shorter than trial findings (Okworo, 2017), a new PHI was proposed, adjusting the PHI from 12 days to 14 days. Reviews on PHI have been preferred instead of decreasing application rates. PHIs reviewed with a targeted action threshold for efficacy and safety derives the Action Pre-Harvest Intervals (APHI) of a subject pesticide (Horská *et al.*, 2020; Kocourek *et al.*, 2017). The post application duration specified on the label PHI defines durations and amount of use of a pesticide while the APHI defines the target threshold based on needs including trade.

Table 2.9.1: Trial results for influence of post application periods (PAP) on diazinon residual levels in selected vegetables.

Country	Vegetable	Conc. (mg/Kg) at PAP			MRL	PHI	References
Thailand	Kale	27.9(0)	0.69(5)	0.05(11)	0.05	14	(Bodzian, 1993)
Finland	Chinese cabbage	2.91 (0)	0.73(5)	0.04(10)	0.05	14	(MIT, 1991)
Bangladesh	Eggplant	2.07(0)	0.73(5)	0.04(10)	0.2	7	(Prodhan <i>et al.</i> , 2018)
Bangladesh	Yardlong bean	2.77(0)	0.51(5)	0.04(10)	0.2	7	(Prodhan <i>et al.</i> , 2018)
Bangladesh	Brinjal	2.23(0)	0.40(5)	0.30(6)	0.5	3	(Kabir <i>et al.</i> , 1970)
Kenya	Kales	49.0(0)	20.40(4)	3.12(11)	0.05	14	(Okworo, 2017)

Notes: Numbers in brackets () are post application days used in trials. Recommended Pre-harvest Intervals (PHI) follows extrapolation action_{PHI} strategy with provisions for time lapse of between one and three days added to the actual post application period at which Codex MRL is realized.

Post application durations are based on the fact that conventional pest management leaves residues in almost all the vegetables as a result of pre-harvest application (Usha & Singh, 2014). Since the residues are chemical contaminants and reduce the medicinal and nutritional values of the vegetables, PHI should be observed to minimize residue quantities (Lintas, 1992; Mwanja *et al.*, 2017).

Studies on PHI observance and residue levels in Bangladesh (Prodhan *et al.*, 2018), Egypt (Hanafi *et al.*, 2018), and Kenya (Mutai *et al.*, 2015; Mwaniki, 2017) confirm that the recommended PHIs are often accurate and safe if observed together with complementing good agricultural practices (Egyptian Ministry for Agriculture and Land Reclamation, 2017). High residue levels when reported are consequences of malpractices contrary to good agricultural practices (GAP) in pesticide application procedures (Kiwango *et al.*, 2018; Mwanja *et al.*, 2017). The malpractices are rare in the developed nations and for export vegetables but commonly reported in developing countries and in locally consumed vegetables (Jallow,

Awadh, Albaho, Devi, & Thomas, 2017a; Kiwango *et al.*, 2018).

While developed nations have banned home and garden applications of diazinon due to documented health and ecological risks associated with its use (USEPA, 2004), developing countries including Kenya have registered it for use in vegetable farming. The smallholder farmers of most developing countries lack technical application knowledge on the value of post application periods and have low to moderate knowledge on pesticides and environmental pollution (Ato, 2011; Boobis *et al.*, 2008; Mwanja *et al.*, 2017; Szpyrka *et al.*, 2015). Given that smallholder farmers are not adequately monitored and may not observe label PHI and APHI even with increased dosages of pesticides (Mwanja *et al.*, 2017), vegetable contamination with diazinon residues may be at high levels. However, there is an information gap as to how the levels of diazinon residues vary with post-application durations of the pesticide in *Brassica oleracea var. acephala* grown at the KOSFIP area of Homa Bay County of Kenya.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out within the Kimira-Oluch Smallholder Farmers Improvement Project (KOSFIP) site. KOSFIP is an irrigation project located in Rachuonyo (Kimira site) and Homa Bay (Oluch site) subcounties of Homa Bay County in Kenya (Figure 3.1.1). KOSFIP lies between latitudes 0° 20' S and 0° 30' S and longitudes 34° 30' E and 34° 39' E with an altitude of 1154 m above sea level along the shores of Lake Victoria.

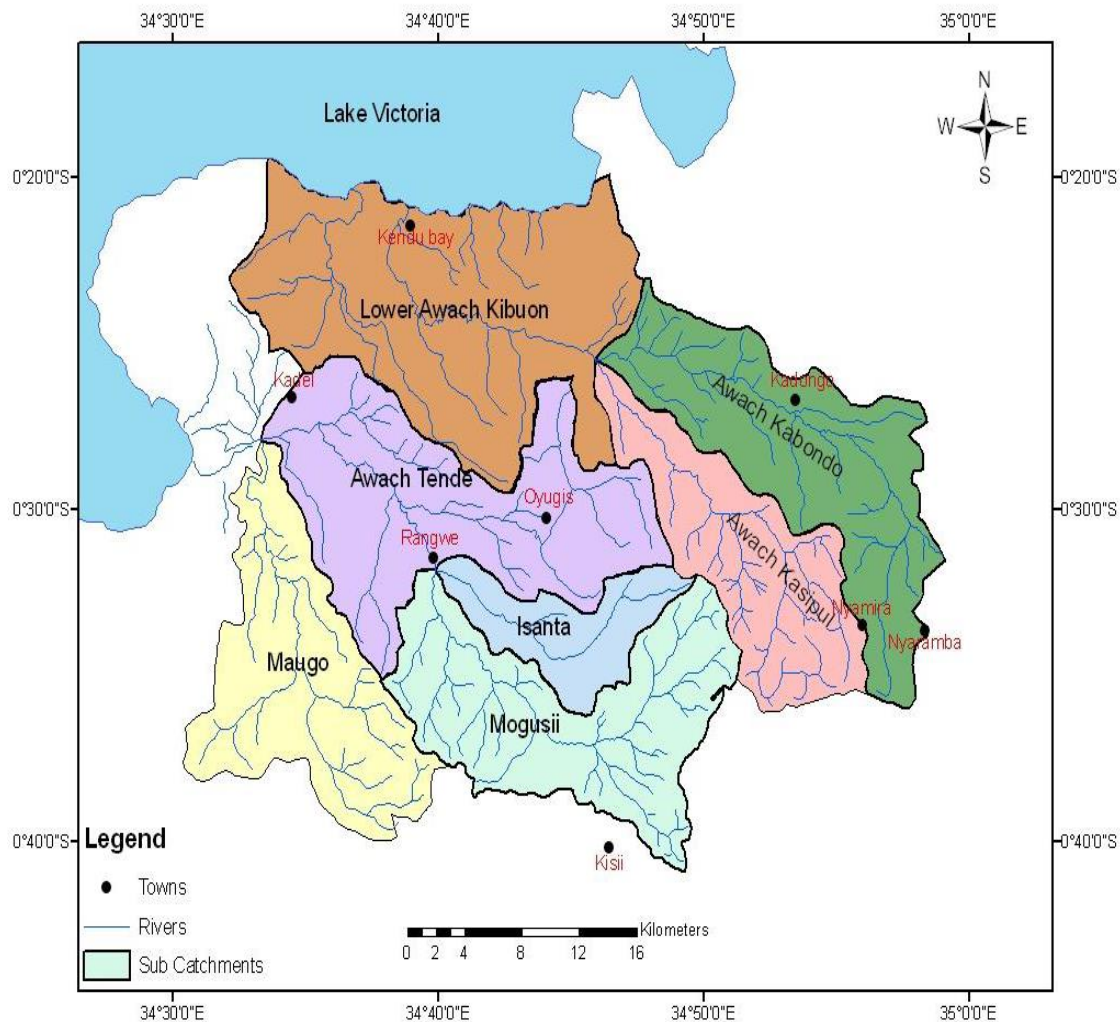


Figure 3.1.1 Map showing the KOSFIP area of Homa Bay County along Lake Victoria Basin, Kenya.

KOSFIP site was preferred for the study given that the introduction of irrigation channels enhanced *Brassica oleracea var. acephala* and other vegetables' production (Government of Kenya & African Development Fund, 2006). In addition, the agro-ecological characteristics of KOSFIP had similarities with locations that promoted rapid growth of vegetables as well as rapid spread of pests and diseases (Cilas *et al.*, 2016; Nurhayati, 2011). The management of such pests would require use of synthetic pesticides. Finally, given that horticulture was replacing fishing as an economic activity at KOSFIP and its environment, proficiency in pesticide applications and safety of produce to consumers of vegetable produce could not have been guaranteed.

Kimira site has a gross area of 1,790 ha out of which 808 ha have been developed into 44 farming blocks whilst Oluch site has a gross area of 1,308 ha but only 666 ha split into 53 farming blocks have been irrigated (Government of Kenya & African Development Fund, 2006). The project area agro-ecological characteristics are sub-humid with mean annual rainfall of between 740 mm and 1200 mm with short and long rainy seasons during April-May and November-December, respectively, and mean annual maximum temperatures of 31 °C and minimum of 18 °C (Government of Kenya & African Development Fund, 2006; Jaetzold *et al.*, 2009). The relative humidity is significantly high ranging between 60 and 75% with potential evapotranspiration rate at 1800mm and 2000 mm per annum. The sites have fertile alluvial soils originating from the nutrient alluvial deposits washed downstream from the rivers and erosions from the Gusii highlands.

3.2 Research design

This study used a combination of cross-sectional survey design for farm-gate baskets and experimental split plot of 8 by 5 factors arranged on a randomized complete block design (RCBD).

3.2.1 Cross sectional survey design.

A cross sectional survey design was used for the first objective of the study. The survey of the farm-gate baskets was done using purposive and snowball sampling techniques to identify at most 40 of the forty-five (45) farms of *Brassica oleracea var. acephala* treated with diazinon within the KOSFIP area. The cross-sectional survey was carried out during the dry season (February to March, 2020) in both Kimira and Oluch sites of the project. During the study period, Kimira site had 18 active blocks with *Brassica oleracea var. acephala* while Oluch had 35 sites. In addition, during sampling, Kimira site had 12 farms meeting the criterion for snowball sampling while Oluch had 33 farms. Of the forty-five (45) farms, 40 were selected based on sample size determination tables (Appendix I) (Krejcie & Morgan, 1970). Kimira had 11 farms while Oluch had 29 farms. The farms were spread equitably to represent Kimira and Oluch sections of the project, taking into consideration all the 97 blocks making the project area. Purposive sampling was biased to farms of *Brassica oleracea var. acephala* treated with diazinon just before the first harvest after planting. The farms identified by the sampling process were tagged for ease of collection. From every sampled farm of *Brassica oleracea var. acephala*, three replicates of 1 Kg freshly harvested leaves of *Brassica oleracea var. acephala* ready for market sales were collected to make 120 samples.

3.2.2 Experimental design.

An experimental field was set up as a split plot randomized complete block design (SP-RCBD) (Oladugba *et al.*, 2013) on a 100 m long by 40 m wide piece of land within the KOSFIP area. The experimental plot was sited within the area of high vegetable production, at latitude 0°27'35"S and longitude 34°33'44"E. The set up was based on the OECD (2011) guidelines for the testing of chemicals for crop field trials. Certified seeds of *Brassica oleracea var. acephala* (Collards Mfalme F1) were purchased from East African Seed Company Limited outlets in Homa Bay Town. The seeds were planted into a nursery for development before transplanting

into the experimental plot. *Brassica oleracea var. acephala* (Collards Mfalme F1) was the most preferred variety in the project because of its presumed resistance to common pests, ability to withstand harsh weather conditions while producing high quality and quantity yields (Edwin Otieno - Awendo Agrovet sales representative, Homa Bay Town personal communication; J, Onyango & L. Atieno– farmers; R, Apodo – Agricultural Extension Officer, personal communication 14/06/2018; L, Cherono & A. Amenity - KOSFIP agronomists, personal communication, 17/06/2018).

3.2.3 Experimental plot layout.

The main treatment plots (Figure 3.2.1) had 160 plants in 32 rows and five (5) columns subdivided into eight sub-plots for harvesting intervals. In between every two successive main plots was a guard row of about 5 m wide with pre-planted maize plants of approximately 1.0 m high at the time of treatments. The seeds of maize planted were variety DK8031 sourced from an agrovet within Homa Bay town. The maize seeds were planted on 15th November, 2019, 60 days before the date of transplanting the vegetables. All recommended agronomic practices for maize crops (KALRO, 2019) including spacing of 75 cm by 30 cm, planting fertilizer (DAP) at 50 Kg/ha, weeding and topdressing with CAN (92 Kg/ha) were observed. The experimental plots were distinctively and conspicuously labeled with details of plot number, date of planting and expected harvesting period. A broader label was erected at the entrance of the plot indicating the variety of vegetable and a caution against consumption of experimental produce “Harmful: not for human consumption”.

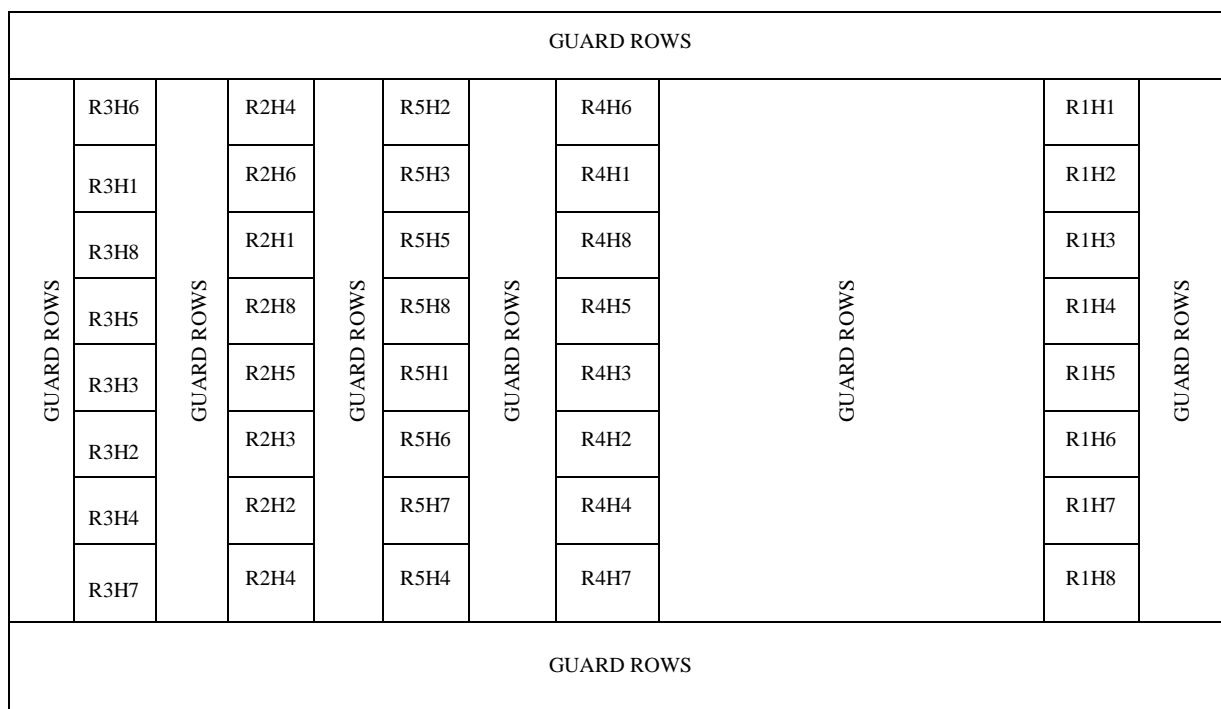


Figure 3.2.1 Experimental field lay-out for one replicate.

Notes: The negative control subplot containing R1 treatments were separated from the rest of the treatments, about 160 metres away, to minimize drift contamination. In between the control subplots and R4 treatments was a larger guardrow of maize plants.

3.2.4 Supplementary preparation of experimental plots.

The regular agronomic practices for *Brassica oleracea var. acephala* production (MoALF/SHEPPLUS, 2019; Sarah & Maina, 2008) were done to all the plants including control experiments. The practices included:

- a) Land preparation (1st & 2nd ploughing) were done at intervals of three weeks (between 10th October 2019 and 3rd November, 2019)
- b) Nursery bed preparations including soil fumigation with imidacloprid (Confidor 70 WG) at label application rate of 1.5 gm⁻² before planting at the seed bed to control in-soil pests that would destroy the seeds and cut germinating seedlings
- c) Watering of seed bed and seedlings before sunrise and after sunset for up to three weeks after planting.

- d) Drenching of planting holes with imidacloprid (Confidor 70 WG) at label application rate of 1.5 gm^{-2} to control in-soil pests. Done 30 days after seed germination to protect transplanted seedlings.
- e) Transplanting seedlings into planting holes spaced out at 60 cm by 40 cm, pre-treated with about 3 g of diammonium phosphate (DAP). Transplanted seedlings were treated immediately with 40 g easygrow starter (NPK – 18-20-21 + TE) in 20 litres of water for faster root development and antistress.
- f) One week after transplanting, the vegetables were treated with 40 g of easy grow vegetative (NPK 27-10-16 + TE) in 20 litres of water to improve leaf development and minimize effect of stressing conditions. The easy grow solution was mixed with 50 g of Dithane M-45 WP (mancozeb)/40 litres of water for control of Downy mildew and dumping off, common *Brassica oleracea var. acephala* fungal infections.
- g) Two weeks after transplanting, weeding was done followed with top dressing with calcium ammonium nitrate (CAN) at 40 Kg/ha. Repeat weeding and top dressing with CAN (at 80 Kg/ha) was done after another three weeks.
- h) Watering of the plants by flood irrigation was done to ground wetness once every three days. Pruning of drying leaves was done on a daily basis to manage spread of infections.

3.2.5 Spray equipment calibration.

The Knapsack Sprayer (Farmate model - 17 litres) was calibrated using stationery method (Chikuwu, n.d.; Mashingaidze *et al.*, 2003) modified for applicator walking speed of 1.2 m/s at 144 runs/ha, sprayer pressure and floodjet nozzle orifice size of 015-F110 for 120 ± 5 litres/ha with swath width of 100 cm. The nozzle was maintained at 0.6 ± 0.05 m above the ground to minimize drift. The calibration was set to evenly spread diazinon insecticide on the leaves.

3.2.6 Treatments of experimental plots.

At the end of the seventh week (50 days) after transplanting, the pesticide Diazol 60EC was sprayed using the calibrated Knapsack sprayer (Farmate 17 litres). Every main block of the vegetables was given separate treatments of different rates with diazinon pesticide (formulation diazol 60 EC). Rate 1 - 0.0 L/ha as negative control; rate 2 - 0.6125 L/ha; rate 3 - 1.25 L/ha recommended rate as positive control; rate 4 - 1.8625 L/ha; and rate 5 - 2.5 L/ha. Post application duration in number of days, randomly chosen within a period of 21 days from time of application, lapsing before harvesting were the sub-plot factor with eight (8) harvesting levels: day 0, day 5, day 10, day 13, day 16, day 18, day 20 and day 21.

3.3 Sample collection

The leaf samples each weighing approximately 1 Kg were collected by the method described by Sajjad *et al.* (2009). The method recommended use of disposable gloves changed every time before collecting the next sample. All samples (from farm – gate baskets and experimental fields) were collected at the same period, packed separately and placed in labeled sterilized plastic zip-lock bags in readiness for preparations, processing and extraction within 24 hours from the time of collection. Dusts from the samples were removed with light brushing using a soft brush without washing prior to placing them into the sample collection bags. The samples were stored in ice-packed cooler boxes at about -4 °C to minimize chances of degradation before processing and analysis.

3.3.1 Sample collection from farm-gate baskets.

Using Sajjad *et al.* (2009) method as described above (Section 3.3), farm-gate samples from tagged farms were collected at the farm-gates before any culinary applications such as washing was done by the vendors. All the samples were collected from the first harvest leaves.

3.3.2 Sample collection from experimental fields.

Approximately 19 of the 20 plants per split plot were randomly sampled based on the Krejcie & Morgan Tables formula (Krejcie & Morgan, 1970). The samples from each leaf were mixed to obtain 1 Kg samples. Each plant averagely provided 53 grams (three leaves). A total of 2,400 plants were available for sampling (800 plants per replicate field).

3.4 Sample processing, preparation, extraction and partitioning

The processing, preparations, extraction and partitioning of the vegetable samples for diazinon residues analysis were carried out using Quick Easy Cheap Effective Rugged and Safe (QuEChERS) multi-residue method (Anastassiades *et al.*, 2003) as adopted and validated by the Analytical Chemistry Laboratories of Kenya Plant Health Inspectorate Services (KEPHIS) method M0326. The details of method M0326 were as outlined in the subsequent Sections 3.4.1 to 3.6.

3.4.1 Sample processing and preparation.

The vegetable samples were coarsely cut with a knife then chopped and homogenized with a Stephan Chopper food processor. About 100 g of the homogenized samples were placed in sample containers and were then stored frozen at $-18\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ in readiness for extraction.

3.4.2 Extraction and partitioning of samples.

A 10.0 ± 0.1 g of the homogenous wet samples and two controls were weighed into 50 mL centrifuge tubes and 50 μL of procedural internal standards, malathion D10 (10 ppm) were added. One of the control samples was fortified with 10 mg/kg diazinon standard solution to achieve the 0.01 mg/kg for LC – MS/MS analysis. To the contents of the centrifuge tube, 10.0 ± 0.2 mL extraction solvent acetonitrile (MeCN) HPLC grade was added, closed and shaken vigorously for 1 minute at 1000 revolutions per minute (rpm) using Geno grinder. The resulting

homogenous mixture in the centrifuge tube was then subjected to liquid-liquid partitioning step using 6.5 g of premixed QuEChERS extraction salts. The extraction salts comprised 4.0 ± 0.2 g magnesium sulphate anhydrous for removal of water and salting out acetonitrile (MeCN); 1.0 ± 0.05 g sodium chloride to increase selectivity of analyte by reducing amount of co-extracted matrix; 1.0 ± 0.05 g trisodium citrate dihydrate and 0.5 ± 0.03 g disodium hydrogen citrate sesquihydrate as a citrate buffer for pH adjustment. The tube was closed and immediately shaken vigorously by hand to avoid caking. The mixture was again shaken by Geno grinder for 1 minute with 1000 rpm then centrifuged for 5 minutes at 3700 rounds per minute (rpm) to allow phase separation of the mixture. An aliquot of 500 μ L of the mixture was transferred into a 2.0 mL vial followed with 495 μ L of HPLC grade water and 5 μ L of injection internal standard dimethoate D6 (10 ppm). The mixture was vortexed to mix for LC-MS/MS analysis.

3.4.3 Preparation of calibration solutions.

Calibration standards were prepared using a control matrix containing no detectable diazinon residues analytes. Using an automatic pipette, 4 mL of control blank was put into a 15 mL centrifuge tube followed with 4 mL of HPLC grade water and vortexed to mix. Reference standard solutions from 10 ppm diazinon were prepared for analysis at concentrations of 0.005 μ g/mL, 0.02 μ g/mL, 0.05 μ g/mL, and 0.2 μ g/mL using 8 mL matrix matched control. The blank, standards, control and extracts were then run using LC – MS/MS (dMRM) mode and quantitatively determined. The units of conversion were thus (Caon, 2018): 1ppm = 1000 ppb = 1 mg/Kg = 1000 μ g/L.

3.5 Instrumentation, method validation and quality control

3.5.1 Instrument specifications.

The extracted samples of *Brassica oleracea var. acephala* were analysed using Agilent Liquid Chromatography triple Quadruple 6460 LC-MS/MS. The column temperature was set at 40.0 °C. The auto-sampler injection and ejection speed were 200 µL/min with an injection volume of 3.00 µL.

3.5.2 Method validation and quality control for diazinon residues in *Brassica oleracea var. acephala*.

The 2018 Standard Operating Procedures (SOP) number M0326 for QuEChERS Multi-Residue Method for Analysis of Pesticides Residues (Anastassiades *et al.*, 2003) in fruits and vegetables high water matrices validated by Analytical Chemistry Laboratory of the Kenya Plant Health Inspectorate Services (KEPHIS), was used in the determination of residue levels. The validation parameters (Raposo & Ibelli-Bianco, 2020) based on SANTE/11813/2017 document which included linearity, limits of detection (LOD) and quantitation (LOQ), trueness and precision were observed (Appendix. J). Calibration curve (Appendix M) was drawn according to analyte ranges of concentration and response to the LC-MS/MS. This was achieved by using five replicates of different concentrations diluted with blank extract samples. Evaluations of accuracy and precision parameters were done by recovery experiments (recovery range of 93 – 123%) in which each analyte standard was spiked with blank *Brassica oleracea var. acephala* slurry in six replicates. The replicates were prepared separately at three different concentrations of 10, 20 and 50 µg Kg⁻¹. Limits of detection (LODs) of each analyte was validated by comparing the signal-to-noise (S/N) ratio magnitude to the background noise obtained from blank sample in the six replicates that presented mean coefficient of variations (CV) of less than 20%. The time window for the signal - to - noise (S/N) ratio was set at $t < 2$

minutes. LOD was calculated using the mathematical expression (Forootan *et al.*, 2017; Rao, 2018):

$$LOD = S/N \text{ ratio} \times CV \times \text{Mean concentration of blank samples} \dots\dots\dots (5)$$

The limits of quantification (LOQs), defined as the minimum concentration of an analyte that can be identified and quantified with 99% confidence, was calculated using the mathematical expression (Forootan *et al.*, 2017; Rao, 2018):

$$LOQ = 10 \times CV \times \text{Mean concentration of blank samples} \dots\dots\dots (6)$$

3.6 Analytical determination of diazinon residues

For quantitative analysis of the analytes, 3 µL of the solvent blank, matrix control, calibration standards and spikes, and sample extracts were injected into the LC-MS/MS instrument. Responses were recorded for both internal standards and samples. A calibration curve of responses against concentration of calibration standards was obtained (Appendix K). The results of concentrations of diazinon residues for all the samples were calculated from responses obtained from the calibration curve (Appendix K). Respective chromatograms and graphics for quantitation and confirmation were obtained. Samples of chromatograms are shown (Appendix M).

3.7 Analysis of residual diazinon concentration in *Brassica oleracea var. acephala* of KOSFIP

3.7.1 Statistical analysis of farm gate samples

The cross-sectional survey residual diazinon data for farm-gate samples were subjected to descriptive statistics to determine the means, standard deviations, quartiles and interquartile range, minimum and maximum values, range of values and the coefficient of variations (CV).

a) Mean residual diazinon concentrations per block

The mean residual diazinon concentrations in the farm gate samples were determined at two levels. The first level was calculating the arithmetic average of the three replicates from each of the forty KOSFIP blocks:

$$\text{Mean per block } (\bar{x}) = (x_1 + x_2 + x_3)/3 \dots\dots\dots (7)$$

The second level involved calculation of the arithmetic average of mean residual diazinon levels obtained from equation (7) and corresponding standard deviations (Std Dev) in all the forty blocks:

$$\text{Mean } (\bar{x}) = (x_1 + x_2 + x_3 + \dots\dots\dots x_{40})/40 \dots\dots\dots (8)$$

b) Quartile distribution

The median and quartiles (lower and upper) were determined using the equations (Milton & Arnold, 1995):

$$\text{Lower quartile } Q_1 = (N+1)/4 \dots\dots\dots (9)$$

$$\text{Median } Q_2 = 2(N+1)/4 \dots\dots\dots (10)$$

$$\text{Upper quartile } Q_3 = 3(N+1)/4 \dots\dots\dots (11)$$

Where *N* is the number of samples under investigation.

$$\text{The interquartile range (IQR) was determined thus: } Q_3 - Q_1 \dots\dots\dots (12)$$

c) Minimum, maximum and range of residual diazinon levels

The minimum and maximum residual diazinon levels were also determined. The minimum residual levels were those below the limits of detection (LODs) of the equipment and were recorded as < 0.01 mg/Kg. In computations, the <0.01 were assigned 0.00 mg/Kg. The maximum residual levels were taken as the greatest value when all data were sorted in ascending order.

The mean residual levels were also compared with Codex MRL values of 0.05 mg/Kg and evaluated for health risk assessment. Residual diazinon levels with health risk index (HRI) > 1.00 were assigned as posing health risks while values of $HRI \leq 1.00$ were marked as not posing health risks to consumers. Range of values was estimated by getting the difference between the maximum residual level and the minimum residual level (the < LOD).

d) Co-efficient of variations

Percentage (%) co-efficient of variations (CV) were computed using the expression:

$$CV (\%) = (\text{Standard deviation}/\text{Mean}) *100 \dots\dots\dots (13)$$

The magnitude of CV (%) predicted the variability of treatments leading to the residual levels of data about the mean (FAO, 2012a). When there is a large range of residue levels indicated by a very high coefficient of variation, different populations of samples may be suspected.

Table 3.7.1: Table showing the interpretation of coefficient of variation

CV (%) Range	Interpretation of CV range
Below 10	Treatments with very high precision and minimum variability
10 -20	Treatments with medium precision and medium level variability
20 – 30	Treatments with low precision and high variability
>30	Treatments with very low precision and very high variability

Adapted from (Marcisz, 2013)

The split - plot design in randomized complete block (RCB) arrangement data on the levels obtained from LC-MS/MS chromatograms was subjected to Statistical Analysis Systems' (SAS) using two-way analysis of variance (ANOVA) and least significant differences (LSDs) to assess significant treatment effects at $P < 0.05$ and to determine variations in the pesticide

residue levels with the application conditions (rates of application and post application duration). ...

e) Residual diazinon health risk assessment in samples

The residual pesticide health risk indices (HRI) estimations for children (HRI_C) and for adults (HRI_A) based on the farm-gate samples were estimated using the European Union formula (European Union, 2005):

$$\text{HRI} = \frac{\text{Estimated Daily Intake (EDI)}}{\text{Acceptable Daily Intake (ADI)}} \dots\dots\dots (14)$$

The EU formula provides that the EDI be determined by multiplying the sample residual pesticide concentration (mg/Kg) by the estimated WHO food consumption rate (Kg/day), and dividing by the number of the estimated WHO average body weight (WHO & FAO, 2009). HRI > 1.0 were considered as posing health risks hence not safe for human health. HRI ≤ 1.0 were considered not posing immediate health risks and thus safe for human health (Darko & Akoto, 2008; WHO, 2005). The average daily vegetable intake for an adult of average weight 60 Kg was considered to be 0.345 Kg/Person/day while children average daily intake was considered to be 0.232 Kg/person/day for average body weight of 10 Kg (Wang *et al.*, 2005). The maximum acceptable daily intake (ADI) was considered to be 0.003 mg/Kg body weight while the acute reference dose (ARfD) was 0.03 mg/Kg body weight (FAO & WHO, 2016b).

3.7.2 Experimental samples.

a) Determination of residual levels of diazinon in *Brassica oleracea var. acephala* at the recommended PHI

The mean residual diazinon residue levels were determined for each rate of application with respect to harvesting periods (0, 5, 10, 13, 16, 18, 20, 21 days). Regression curves were then drawn based on the first order kinetics model given by Equation (1) Section 2.4.5. From the

regression curve for each rate of application, the corresponding C_t for $t = 12$ days (PHI) was read from the curve and the outcome was compared to the Codex MRL (0.05 mg/Kg)

b) Determination of post application periods (APHI) for different rates of application at which the MRL is achieved

The effect of post application period on the residual diazinon levels in *Brassica oleracea var. acephala* were tested with relative persistence (%). The initial concentration of the residual diazinon for each rate were considered as 100% persistence. With increasing post application periods, the relative persistences were determined as a fraction of the initial concentration (Abdel-Hamid & El-Hefny, 2017). Since the MRL of diazinon in *Brassica oleracea var. acephala* was set at 0.05 mg/Kg, relative persistences were extended to at least the percentage giving residual levels \leq MRL within the experimental period. The RL_{50} (days) values were calculated from Equation (1) Section 2.4.5 by finding the value of t for which C_t was equivalent to 0.05 mg/Kg.

Similar to Section 3.7.2 (a), the post application periods (t days) at which the residual diazinon levels were \leq MRL (0.05 mg/Kg) were read from the regression curves for each rate of application and compared to the recommended pre-harvest interval (PHI). The values read from the curves were rounded off to the nearest whole number days and increased by 1 day to establish the effective action PHI at which the residual levels were within the Codex MRL (FAO, 2012a).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Method validation and quality control.

4.1.1 Linearity.

The calibration curve linearity was determined with diazinon standards diluted in HPLC grade water as solvent for series 0.005 µg/mL, 0.01 µg/mL 0.02 µg/mL 0.05 µg/mL 0.075 µg/mL 0.1 µg/mL and 0.2 µg/mL. The curve was obtained by plotting analyte concentration (mg/Kg) versus the peak areas. The resultant calibration curve (Appendix K) equation was given by

$$y = 22.593273x + 0.219378 \dots\dots\dots (15)$$

Where y was the relative response peak area while x was the relative analyte concentration in mg/Kg. The correlation coefficient (R^2) was 0.98698022.

4.1.2 Limits of quantification (LOQ).

The lowest validated residual diazinon levels with acceptable precision and trueness limits of quantification was 0.03 mg/Kg for LC – MS/MS analysis in *Brassica oleracea var. acephala*. Based on SANTE/11813/2017 guidelines on analytical quality control and validation procedures for pesticide residues analyses in food and feed, LOQ values are acceptable for $LOQ \leq MRL$.

4.1.4 Trueness and precision.

The mean of recovery (trueness) was tested with 6 replicates at 3 fortification levels of 10 ppb, 20 ppb and 50 ppb by spiking 10 g of blank samples with diazinon standard solutions. Mean recoveries for 10 ppb ranged from 116.56% to 131.16%. This range exceeded the acceptable range (70% - 120%) provided by SANTE/11813/2017. However, since the range was consistent for the concentration, the trueness and precision for 10 ppb was acceptable. Mean recoveries for 20 ppb ranged from 91.105% to 96.255%. The trueness of the output data was within the SANTE/11813/2017 specifications. Mean recoveries for 50 ppb ranged between 83.844 and 101.454%. the trueness was equally within the SANTE/11813/2017 specifications. Overall, the recovery standard deviation (RSD) ranged from

0.0615 to 0.2515 for LC – MS/MS. Based on SANTE/11813/2017 guidelines on analytical quality control and validation procedures for pesticide residues analyses in food and feed, the mean recoveries and corresponding standard deviation were within the acceptable range of 70 - 120%. The repeatability precision that involved repeat of recovery levels (0.001 – 0.005) mg/Kg with 6 replicates for each level per day ranged from 3.476% to 9.734%. The range was within the acceptable range $\leq 20\%$. Consequently, the performance parameters provided by the SANTE/11813/2017 confirm that QuEChERS sample preparation method coupled with LC – MS/MS quantitative analysis was appropriate and suitable for residual diazinon determination in *Brassica oleracea var. acephala*.

4.1.3 Limits of detection (LOD).

The results for instrument optimization (Appendix L) and validation and quality control of equipment output (Appendix J) for QuEChERS method M0326 adapted by the Analytical Chemistry Laboratories of Kenya Plant Health Inspectorate Services (KEPHIS) met the acceptable standards for analysis of pesticide residues. The results of residual diazinon levels in *Brassica oleracea var. acephala* of KOSFIP were therefore highly accurate and reliable. The limits of detection (LODs) and limits of quantification (LOQs) were established at 0.01 mg/Kg and 0.03 mg/Kg, respectively.

4.2 Quantification of levels of diazinon residues in the farm-gate baskets of *Brassica oleracea var. acephala* from the KOSFIP area of Homa Bay County for health risk assessment

Results for analysis of diazinon residues in the farm-gate samples (Table 4.2.1) showed that 92.5% of all the samples had detectable levels of residual diazinon while 7.5% were non-detectable. Within the samples with detectable levels, 67.57% were above the Codex MRL of 0.05 mg/Kg while 32.43% were below the Codex MRL.

Table 4.2.1: Levels of diazinon residues in farm-gate baskets of *Brassica oleracea* var. *acephala* from selected KOSFIP blocks and resultant EDI and HRI for children and adults.

KOSFIP BLOCK	Mean Conc. (mg/Kg)	EDI _C	EDI _A	HRI _C	HRI _A
1	0.0310 ± 0.0378	0.0007	0.0002	0.2397	0.0594
2	< LOD ± 0.0000	0.0000	0.0000	0.0000	0.0000
3	0.8201 ± 0.9989	0.0190	0.0047	6.3421	1.5719
4	0.0505 ± 0.0615	0.0012	0.0003	0.3905	0.0968
5	1.0647 ± 1.2969	0.0247	0.0061	8.2337	2.0407
6	0.0476 ± 0.0580	0.0011	0.0003	0.3681	0.0912
7	0.0529 ± 0.0644	0.0012	0.0003	0.4091	0.1014
8	0.0161 ± 0.0196	0.0004	0.0001	0.1245	0.0309
9	< LOD ± 0.0000	0.0000	0.0000	0.0000	0.0000
10	0.0610 ± 0.0743	0.0014	0.0004	0.4717	0.1169
11	0.8281 ± 1.0087	0.0192	0.0048	6.4040	1.5872
12	0.0950 ± 0.1157	0.0022	0.0005	0.7347	0.1821
13	0.0476 ± 0.0579	0.0011	0.0003	0.3681	0.0912
14	0.0558 ± 0.0680	0.0013	0.0003	0.4315	0.1070
15	0.1465 ± 0.1784	0.0034	0.0008	1.1329	0.2808
16	0.4039 ± 0.4920	0.0094	0.0023	3.1235	0.7741
17	0.0295 ± 0.0359	0.0007	0.0002	0.2281	0.0565
18	0.0226 ± 0.0275	0.0005	0.0001	0.1748	0.0433
19	0.0297 ± 0.0362	0.0007	0.0002	0.2297	0.0569
20	0.6471 ± 0.7882	0.0150	0.0037	5.0042	1.2403
21	0.9460 ± 0.9216	0.0219	0.0054	7.3157	1.8132
22	0.0761 ± 0.0861	0.0018	0.0004	0.5885	0.1459
23	0.0156 ± 0.0192	0.0004	0.0001	0.1206	0.0299
24	0.0913 ± 0.1111	0.0021	0.0005	0.7061	0.1750
25	0.0433 ± 0.0527	0.0010	0.0002	0.3349	0.0830
26	0.2013 ± 0.2454	0.0047	0.0012	1.5567	0.3858
27	0.5408 ± 0.0452	0.0125	0.0031	4.1822	1.0365
28	< LOD ± 0.0000	0.0000	0.0000	0.0000	0.0000
29	0.0495 ± 0.0602	0.0011	0.0003	0.3828	0.0949
30	0.1166 ± 0.1421	0.0027	0.0007	0.9017	0.2235
31	0.0431 ± 0.0525	0.0010	0.0002	0.3333	0.0826
32	0.6561 ± 0.7992	0.0152	0.0038	5.0738	1.2575
33	0.4434 ± 0.5411	0.0103	0.0025	3.4290	0.8499
34	1.0549 ± 0.8949	0.0245	0.0061	8.1579	2.0219
35	0.0599 ± 0.0370	0.0014	0.0003	0.4632	0.1148
36	0.7413 ± 0.9029	0.0172	0.0043	5.7327	1.4208
37	0.9969 ± 1.2143	0.0231	0.0057	7.7094	1.9107
38	0.0415 ± 0.0505	0.0010	0.0002	0.3209	0.0795
39	0.4122 ± 0.5022	0.0096	0.0024	3.1877	0.7901
40	0.6192 ± .7443	0.0144	0.0036	4.7885	1.1868

Replicates per block = 3; EDI_A – Expected Daily Intake for adults; EDI_C – Expected Daily Intake for children; HRI_A – Health Risk Index for adults; HRI_C – Health Risk Index for children; LOD - Limits of Detection (0.0100 mg/Kg); 7.5% ≤ LOD ≤ 92.5%; MRL ≥ 57.5%

The data set (Table 4.2.1) had no outliers and were all statistically significant. Corresponding measures of central tendency and dispersion derived from the data set are shown in Table 4.2.2.

Table 4.2.2: Measures of central tendency and dispersion for levels of diazinon residues in farm-gate baskets of *Brassica oleracea* var. *acephala* from KOSFIP area and resultant EDI and HRI for children and adults.

Measure of Central Tendency & Dispersion	Mean Conc (mg/Kg)	EDI _C	HRI _C	EDI _A	HRI _A
Mean	0.2900 ± 0.3532	0.0067 ± 0.0082	0.0017 ± 0.0020	2.2424 ± 2.7314	0.5558 ± 0.6770
Minimum	<0.0100	0.0000	0.0000	0.0000	0.0000
1 st Quartile	0.0427	0.0010	0.0002	0.3302	0.0818
Median	0.0686	0.0016	0.0004	0.5301	0.1314
3 rd Quartile	0.5604	0.0130	0.0032	4.3338	1.0741
Inter Quartile R	0.5177	0.0120	0.0030	4.0035	0.9923
Maximum	1.0647	0.0247	0.0061	8.2337	2.0407
Range	1.0647	0.0247	0.0061	8.2337	2.0407
Max Outliers	1.3370	0.0310	0.0077	10.3391	2.5625
CV (%)	121.805	121.805	121.805	121.805	121.805

EDI_A – Expected Daily Intake for adults; EDI_C – Expected Daily Intake for children; HRI_A – health Risk Index for adults; HRI_C – Health Risk Index for children; Mean conc. of 0.0000 represents conc. below Limits of Detection. Since all values fall below the maximum outlier value, there were no outliers and all data were used in the interpretation.

The measures of central tendency (mean, median and mode) displayed a positively skewed distribution (Figure 4.2.1) with a coefficient of variation (CV) of 121.805% (Table 4.2.2). The distribution displayed by the box plot (Figure 4.1.2) suggested that the treatments associated with diazinon use on *Brassica oleracea* var. *acephala* in the study area were highly variable and unregulated. Given that food safety standards encourage infinitesimal residual levels of pesticide residues, a positively skewed distribution with all measures of dispersion falling below Codex MRL would be most preferable. However, the results display mean, median, 3rd quartile and maximum residual diazinon concentrations higher than Codex MRL. Consequently, most of the farm gate *Brassica oleracea* var. *acephala* treated with diazinon at KOSFIP are therefore not safe for human consumption. The findings compare similarly to the levels reported in some vegetables of Bangladesh (Alam *et al.*, 2015), Kuwait (Jallow, Awadh, Albaho, Devi, & Thomas, 2017b), Nigeria (M. Musa *et al.*, 2015), Ghana (Ato, 2011)

and Sudan (Awad *et al.*, 2018), where over 30% of samples reported residue levels above the Codex MRLs.

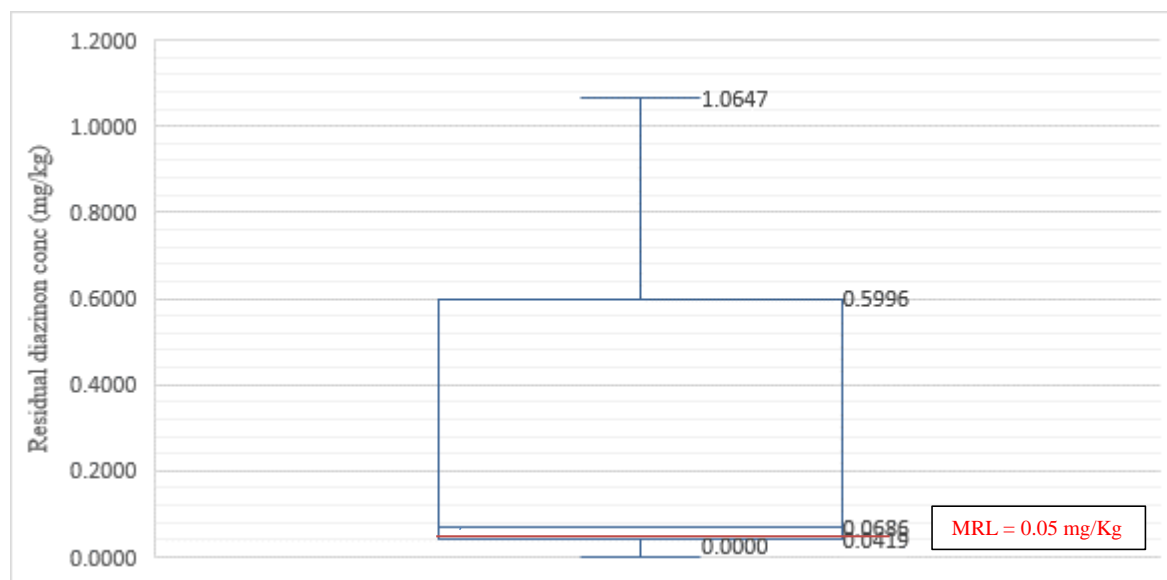


Figure 4.2.1: Box plot showing the distribution of diazinon residue concentrations in farm-gate samples of *Brassica oleracea var. acephala*.

Notes: Approximately 92.5% of samples had detectable residual diazinon levels with 67.57% of the detectable being above the Codex maximum residue limits (MRL) of 0.05 mg/Kg.

The findings were comparatively higher than levels reported by studies done in Ghana (Bempah *et al.*, 2012) and Nigeria (Njoku *et al.*, 2017) and other parts of Kenya (Karanja *et al.*, 2012; Ngolo *et al.*, 2019), which reported trace levels of diazinon residues with less than 10% of the samples being above the Codex MRL. Since the study areas with similar results were of developing nations, the result could be associated to inadequate training of farmers on good agricultural practices and poor surveillance by respective authorities (WHO & FAO, 2019). Consequently, farmers in KOSFIP area require regular surveillance and training on use of diazinon. The findings also suggest that the recommended diazinon application conditions of rates and pre-harvest intervals may not be suitable for the study area. In addition, the findings suggest that KOSFIP farm-gate vegetables need thorough washing before cooking to reduce the leaf surface residual levels on the vegetables.

The determinations of health risk indices (HRI) from the samples (Table 4.2.1) were based on Equation (14). The corresponding data for health risk indices represented by boxplot (Figure 4.2.2) displays a positively skewed distribution where the minimum, 1st quartile and median residual diazinon concentrations in *Brassica oleracea var. acephala* of KOSFIP are less than the no-risk-threshold (HRI = 1.000).

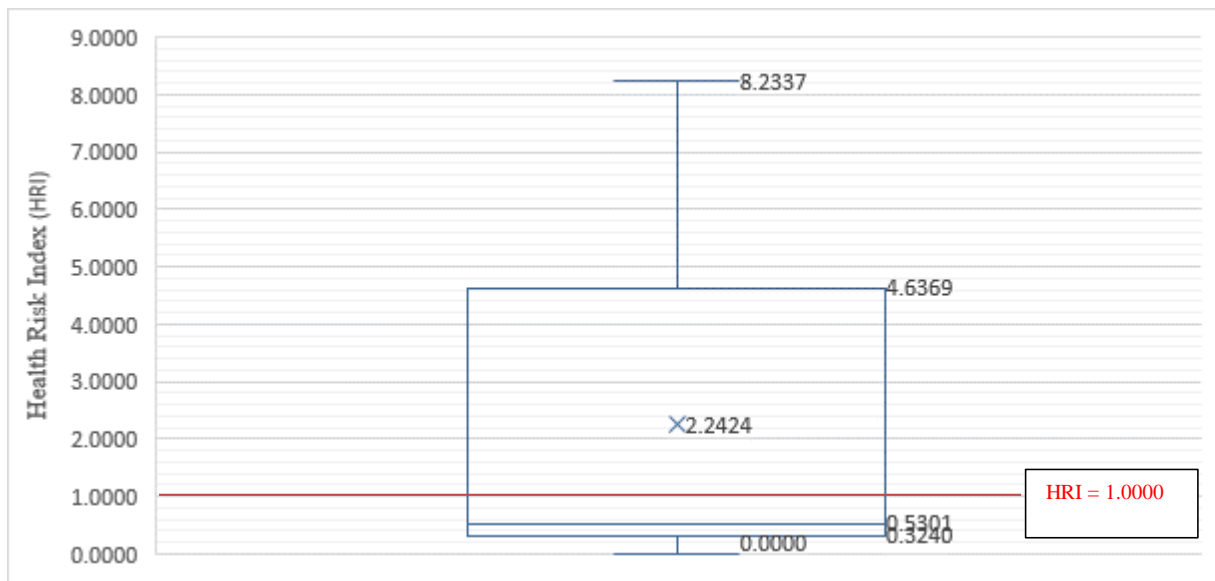


Figure 4.2.2: Box plot showing the dispersion of health risk indices (HRI_c) for children for farm gate *Brassica oleracea var. acephala* of KOSFIP area.

Notes: Distribution positively skewed with approximately 40% of the samples presenting health risk indices (HRI) > 1.00 with a co-efficient of variation (CV) of 121.805%.

However, the mean, 3rd quartile and maximum residual concentrations were approximately 200%, 460% and 823%, respectively, above the safety threshold. This finding confirms that consuming *Brassica oleracea var. acephala* of KOSFIP have a risk probability of about 40%. The estimated daily intake (EDI_c) and resulting health risk indices for children (HRI_c) indicate that children consuming the vegetable from the study area have higher chances of developing diazinon - related health problems (Hancock *et al.*, 2008; Kishi, 2005). Given that data on

residual levels with computed EDI and HRI for children are not available, comparisons of health risk factors for children in different regions have not been documented.

On the other hand, the estimated daily intake for adults (EDI_A) and resultant health risk index for adults (HRI_A) were comparatively lower than ratios for children (Table 4.2.1 and Table 4.2.2). The box plot (Figure 4.2.3) represents a positively skewed distribution of the data set for health risk indices for adults. The positively skewed distribution shows that the minimum, 1st quartile, median and mean residual diazinon concentrations in *Brassica oleracea var. acephala* of KOSFIP were all below the no – risk threshold risk index (HRI = 1.000). The findings were similar to residual levels of diazinon in cauliflower of Bangladesh (Sultan *et al.*, 2016), tomatoes of Spain (Fenoll *et al.*, 2007) and Iran (Rohani *et al.*, 2017), respectively. The effect of the residual levels of diazinon on EDI and HRI for adults were higher than the findings in Chinese kale (Wanwimolruk *et al.*, 2015), spring onion, parsley onion and ginger vegetables in Thailand (Sapbamrer & Hongsibsong, 2014).

In addition, the findings were also higher than results reported for yard long bean in Bangladesh (Sultan *et al.*, 2016), apple in Pakistan (Asi, 2003), *T. occidentalis* and *C. argentea* in Nigeria (Njoku *et al.*, 2017), *Brassica oleracea var. acephala* (Karanja *et al.*, 2012; Ngolo *et al.*, 2019) and tomatoes (Ngolo *et al.*, 2019) in Kenya. Conversely, the findings and the resultant EDI and HRI were lower than ratios for eggplant and tomatoes in Pakistan (Alam *et al.*, 2015; Latif *et al.*, 2011).

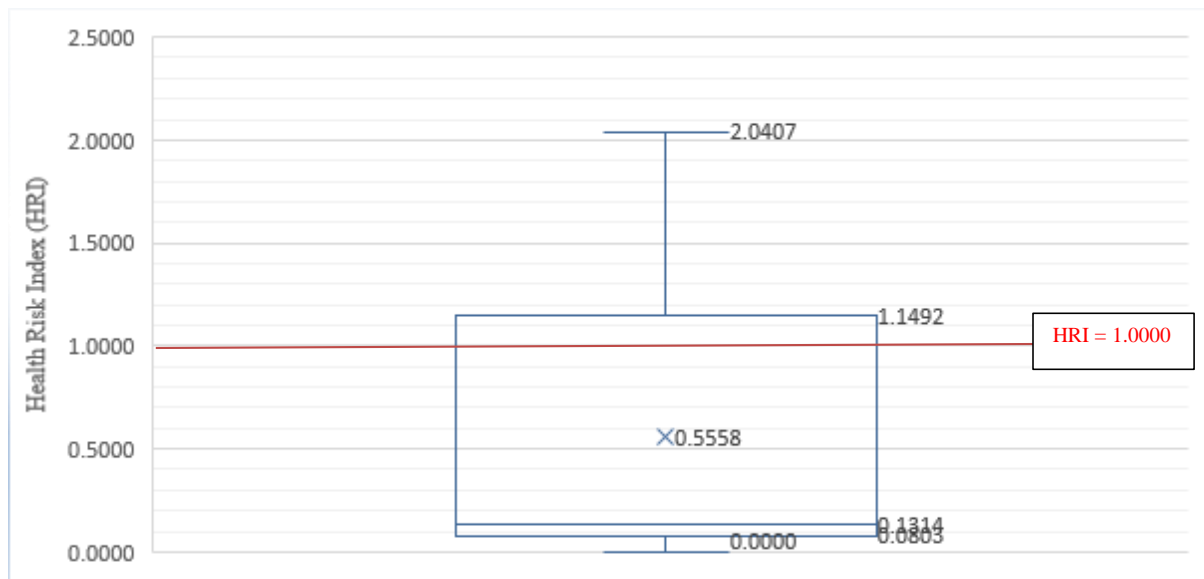


Figure 4.2.3: Box plot showing the dispersion of health risk indices (HRI_A) for adults from farm gate *Brassica oleracea var. acephala* of KOSFIP area.

Notes: Distribution is positively skewed with approximately 28% of the samples presenting health risk indices (HRI) > 1.00.

Similarly, the ratios were below the findings of levels in watermelon (M. Musa *et al.*, 2015), spinach and onions (Akan, Jafiya, *et al.*, 2013) in Nigeria. Other findings with higher than the results for this study included levels in tomatoes in Ghana (Bempah *et al.*, 2012), cucumber and tomatoes in Sudan (Awad *et al.*, 2018). Given the high variability displayed by the coefficient of variation (C.V) of 121.805%, the farmers and consumers of the vegetables are likely to be exposed to diazinon associated health risks (Jallow, Awadh, Albaho, Devi, & Thomas, 2017a). These health challenges may threaten human population in the study area. On the same note, though the adults have a lower mean ratio of 0.559, continuous consumption of this popular vegetable may cumulatively raise the exposure levels and result in devastating health impacts (Savage *et al.*, 1988). The presence of inappropriate levels of diazinon residues in the leaves of *Brassica oleracea var. acephala* may be a consequence of non-adherence to good agricultural practices (GAPs) such as failing to observe the application conditions of

dosage and pre-harvest intervals. Such disregard are popular with smallholder farmers in developing countries for locally consumed vegetables (FAO, 2007c, 2010a; Kiwango *et al.*, 2018) and in regions where surveillance and monitoring activities are inadequate.

The unacceptable residue levels could be due to inability of the farmers to interpret the rates as prescribed on the labels or utter disregard of the rates, and inadequate surveillance of farmers on the use of pesticides. Inappropriate levels may also be attributed to inability of the farmers in the study area to consider other viable methods of pest control provided by Integrated Pest Management (IPM) guidelines (Jallow, Awadh, Albaho, Devi, & Thomas, 2017b; Zyoud *et al.*, 2010) during heavy infestations. To manage the unacceptable residue levels and related health problems, training of farmers on GAP and alternatives to chemical pest control should be initiated in the study area. National regulatory agencies should equally strengthen surveillance on farmers with emphasis on observance of pre-harvest intervals and pesticide dosages. The general public should also be sensitized on the need to reduce the risks through proper culinary processing of the vegetables before consuming them. Lastly, the inappropriate residue levels may result from label application conditions which were extrapolated from field trials done in other regions but not suitable for the study area. It is therefore well justified to constantly monitor residual levels of diazinon in the vegetable and to develop pesticide safety level monitoring policies to mitigate resultant health problems due to unacceptable residue levels.

4.3 Determination of influence of rate of application on residual levels of diazinon in *Brassica oleracea var. acephala* of KOSFIP area at recommended pre-harvest interval

Analysis of results for the field treatments of *Brassica oleracea var. acephala* at the KOSFIP area with diazinon (Table 4.3.1) showed that dissipation for each rate of application presented significantly different diazinon residues levels (at 95% confidence level and $p \leq 0.0001$) at the recommended pre-harvest interval (PHI) of 12 days. The results displayed a co-efficient of

variation of 4.72%, proposing that variability in diazinon dissipation observed on *Brassica oleracea var. acephala* at KOSFIP area could significantly be attributed to differences in rates of application of the pesticide. The findings corresponded with previous trials done with kale in Thailand (MIT, 1991), Japan (Kono & Yamasita, 1974), Germany (Bodzian, 1993), USA (Guth, 1978), Italy (Guth, 1972) and Switzerland (Laanio *et al.*, 1972). In these studies, dissipation trends for different rates of application were similar but displayed variations in residual levels with sites of study.

Table 4.3.1: Diazinon residue levels (mg/Kg) in *Brassica oleracea var. acephala* for application rates and post application periods.

PAP	Rates of Diazinon Application (Liters/Ha)					Mean PAP
	0.0000	0.6125	1.2500	1.8625	2.5000	
0	< LOD ±0.0000	2.5523 ±0.2249	7.987 ±3.0081	10.4409 ±1.4165	14.141 ±1.7927	8.7803 ±4.8613
5	< LOD ±0.0000	0.1082 ±0.0223	0.2826 ±0.0310	0.5450 ±0.0936	0.9028 ±0.1074	0.4597 ±0.3457
10	< LOD ±0.0000	0.0739 ±0.0376	0.1432 ±0.0570	0.3614 ±0.0483	0.7137 ±0.0760	0.3231 ±0.2878
13	< LOD ±0.0000	0.0301 ±0.0166	0.1106 ±0.0409	0.1636 ±0.0506	0.3369 ±0.1127	0.1603 ±0.1299
16	< LOD ±0.0000	0.0100 ±0.0093	0.0158 ±0.0096	0.0557 ±0.0171	0.2282 ±0.0542	0.0770 ±0.1029
18	< LOD ±0.0000	< LOD ±0.0000	0.0100 ±0.0117	0.0330 ±0.0076	0.1309 ±0.0320	0.0427 ±0.0605
20	< LOD ±0.0000	< LOD ±0.0000	< LOD ±0.0000	0.0187 ±0.0039	0.0996 ±0.0297	0.0296 ±0.0475
21	< LOD ±0.0000	< LOD ±0.0000	< LOD ±0.0000	0.0155 ±0.0079	0.0730 ±0.0250	0.0221 ±0.0347
Mean Rate	< LOD ±0.0000	0.3466 ±0.8921	1.0683 ±2.7973	1.4542 ±3.6362	2.0783 ±4.8835	
P≤0.05			≤0.0001			
LSD			0.0379			
CV(%)			4.72			0.0585

CV (%) – Percentage co-efficient of variation; LSD – Least Significant Differences; PAP – Post Application Period (days); Replicates = 4:

The variations in diazinon levels (Table 4.3.1) indicated that extrapolation of residual levels may not be applicable to sites with dissimilar environmental conditions. Inconsistent

dissipation patterns with diazinon have equally been reported for different types of vegetables including eggplant, country bean and cauliflower vegetables (Islam *et al.*, 2014), and in cucumbers (Cengiz *et al.*, 2006). The comparative inconsistencies in dissipation trends were associated with dissimilar phenological stages and morphological variations of the vegetables. From Table 4.3.1, residual levels of diazinon in *Brassica oleracea var. acephala* of KOSFIP at the recommended PHI of 12 days have been computed (with $r^2 > 0.92$) from each application rate regression model equation (Table 4.2.2).

Table 4.3.2: Influence of rate of diazinon application on residual concentrations at PHI on *Brassica oleracea var. acephala* of KOSFIP area.

Rate (Litres/ha)	Linear regression equation	r^2	Concentration (mg/Kg) at PHI (C_t)
0.0000	$y = 0$	N/A	-
0.6125	$y = 1.46e^{-0.32x}$	0.9202	0.0314
1,2500	$y = 4.6683 e^{-0.35x}$	0.9312	0.0700
1.8625	$y = 5.7412e^{-0.287x}$	0.9621	0.1834
2.500	$y = 6.732e^{-0.219x}$	0.9275	0.4862

Notes: Replicates = 4; Rates of application with residual levels > Codex MRL (0.05 mg/Kg) at PHI may pose immediate health risks to consumers. PHI – Pre-harvest Interval.

Comparing experimental results for effect of application rates on residual levels of diazinon at PHI ($t = 12$ days) when different rates of application were used (Table 4.3.2) with the farm-gate survey results (Table 4.2.1), it may be concluded that most smallholder farmers using diazinon on *Brassica oleracea var. acephala* within KOSFIP area may not be observing recommended application rates. The findings point to possible pesticide use malpractices related to rates of application at KOSFIP area. *Brassica oleracea var. acephala* of KOSFIP treated with 0.6125 litres/ha of diazinon had residual levels of diazinon below MRL and posed no immediate health risk to consumers. From the farm-gate survey results, 22.5% of the farmers may have used rate ≤ 0.6125 litres/ha. However, due to low concentration of the active ingredient (ai), the target pests may not be controlled but are likely to develop resistance to

diazinon. Similarly, *Brassica oleracea var. acephala* of KOSFIP treated with the recommended rate of application (1.25 litres/ha) reported residual levels above the MRL at PHI. From the farm-gate survey results (Table 4.2.1), about 27.5% of farmers of the vegetable at KOSFIP may have used this rate of application. Since the corresponding residual levels for rate 1.25 litres/ha were above the Codex MRL and may pose immediate health risks to the consumers, a review of the PHI for the recommended rate may be required to minimize residue levels and corresponding health risks to consumers.

Rates of diazinon application > 1.25 litres/ha also resulted in unacceptable residual levels of diazinon at PHI. While rates higher than 1.25 litres/ha may be due to deliberate overdose during heavy pest infestation, it may also be indicative of lack of knowledge on interpreting the application rates as provided by GAPs and GPAPs. Rate 1.8625 litres/ha had residual values matching farm-gate survey results (Table 4.2.1) for about 12.5% of the farmers at KOSFIP. Experimental double rate of application, 2.5 litres/ha had the highest diazinon residual levels at PHI, with levels matching about 10% of the farm-gate survey results (Table 4.2.1). However, 27.5% of farmers of *Brassica oleracea var. acephala* may have used rates of application higher than 2.5 litres/ha with resultant residual levels ranging between 0.5 mg/Kg and 1.06 mg/Kg. The anticipated long residual period of the broad-spectrum insecticide for rates of application >1.25 litres/ha may enhance pest resistance, immediate acute and chronic health risks to consumers and increased toxicological effects on the general environment. To mitigate the effect of use of higher rates of application on the environment, training of farmers on good agricultural practices and surveillance on pesticide use may be required. In addition, when heavy pest infestations may require higher rates of diazinon application and frequencies, multiple applications of recommended rates and review of post application periods through action PHIs may be required of all farmers.

4.4 Determination of effect of post application duration on the levels of diazinon in *Brassica oleracea var. acephala* of KOSFIP area at different rates of application

Analysis of results for residual levels of diazinon on the leaves of *Brassica oleracea var. acephala* of KOSFIP area (Table 4.3.1) show that diazinon residues persistence for different rates of application declined with increasing post application periods (Table 4.4.1).

Table 4.4.1: Relative persistence (%) of diazinon residues for varied rates of application with post application periods (PAP).

PAP (Days)	Rates of Diazinon Application (Liters/ha)			
	0.6125	1.25	1.8625	2.5
0	100.00	100.00	100.00	100.00
5	4.24	3.54	5.22	6.38
10	2.90	1.82	3.46	5.05
13	1.18	1.39	1.57	2.38
16	0.33	0.20	0.53	1.61
18	0.00	0.09	0.32	0.93
20	0.00	0.00	0.18	0.70
21	0.00	0.00	0.15	0.52

Notes: Rates of application > 1.25 litres/ha resulted in persistence of diazinon beyond the experimental period. The MRL values were achieved at 1.96%, 0.63%, 0.48% and 0.35% of initial residue concentrations at 0 days after one application (2 hrs after application) for 0.6125 litres/ha, 1.25 litres/ha, 1.8625 litres/ha and 2.5 litres/ha, respectively. Safe waiting periods were achieved by rates 0.6125 litres/ha, 1.25 litres/ha and 1.8625 litres/ha within the experimental period while rate 2.5 litres/ha was beyond the experimental period.

Subsequently, the levels of diazinon residues were significantly ($p < 0.05$) different with post application periods. The variability of diazinon residue levels with a coefficient of variation (CV) of 4.72% suggests that post application periods defined by PHI is a significant application condition for diazinon in *Brassica oleracea var. acephala* at KOSFIP area. Diazinon being a non-systemic endolytic insecticide, its dissipation between day zero (0) and day five (5) after

application, was rapid for all rates of application. This could be due to high vapour pressure (9.1×10^{-5} mmHg) (Table 2.6.1) and resultant high volatility of the molecule. Persistence between five (5) days and 21 days were comparatively lower than day zero to five. This could be due to slow chemical degradation and microbial metabolism within the plant cuticle. The results were similar to research findings with diazinon and other organophosphate insecticides on tomatoes (Prieto *et al.*, 2002), olives and olive oil (Cabras *et al.*, 1997), turfgrass (Kuhr & Tashiro, 1978), peach fruit (Minelli *et al.*, 1996), gerbera (Hatzilazarou *et al.*, 2005) and varieties of speciality vegetables (Braun *et al.*, 1980; Ripley *et al.*, 2003). Differences in the persistence were observed with diazinon presenting higher residual levels than other organophosphates on different vegetables. This observation could be due to differences in plant leaf characteristics. *Brassica oleracea var. acephala* has broad leaves which may hasten rapid initial dissipation but equally retain the pesticide in its cuticles.

Variability in the persistence of diazinon with rates of application suggested that the approximate waiting periods for residual levels to match the recommended MRL of 0.05 mg/Kg increased with rate of application. Consequently, the suggested values of action PHIs (t_{APHI}) (safe days after application) for each rate's dissipation model with the Codex MRL as the residue concentration at time t (C_t) value established the rate-specific action pre-harvest interval (APHI) (Table 4.4.2).

Table 4.4.2: Summary of computed RL_{50} and suggested Action Pre-Harvest Intervals $_{0.05}$ for one application of various rates of application.

Rate (Litres/ha)	Linear regression equation	r^2	K day^{-1}	RL_{50} (days)	MRL achieved at (PAP) days	Suggested APHI(days)
0.0000	$Y = 0$	N/A	N/A	N/A	-	-
0.6125	$Y = 1.46e^{-0.32x}$	0.9202	0.320	2.17	10.55	12
1,2500	$Y = 4.6683 e^{-0.35x}$	0.9312	0.350	1.98	12.96	14
1.8625	$Y = 5.7412e^{-0.287x}$	0.9621	0.287	2.42	16.53	18
2.500	$Y = 6.732e^{-0.219x}$	0.9275	0.219	3.16	22.39	23

Notes: MRL – Maximum Residue Limit; PAP – Post Application Period; PHI – Pre-harvest Interval; RL_{50} : Residual half-life. The action pre-harvest intervals ($APHI_{0.05}$) for each rate of application were arrived at by rounding the Post Application Period (PAP) at which the MRL (0.05 mg/Kg) was achieved to the nearest whole number days and adding 1 day (FAO, 2012a). The rate of dissipation (k^{-day}) displays dissipation efficiency for each rate of application. While “k” values were consistently declining implying decreasing efficiency with increasing rates of application, an inconsistency was noted for the recommended rate of application.

The safe post application periods corresponding to the Codex MRLs are all different and increasing with rates of application. The computed residual life-times (RL_{50}) of different rates of application of diazinon on *Brassica oleracea var. acephala* of KOSFIP area compared differently to RL_{50} values for different vegetables in other study locations. For rate 0.6125 litres/ha, the RL_{50} value of 2.17 days corresponded to 2.25 days for *Brassica oleracea alboglabra cv. Guy Lon* (Ripley *et al.*, 2003) and 2.2 days for *Cichorium endivia* (Willis & McDowell, 1987). These comparable values were marginally equivalent to the standard RL_{50} of diazinon in plant matrices (2.4 days) (Lewis *et al.*, 2016). The findings of Ripley *et al.*, (2003) and Willis & McDowell (1987), though comparable to the results for rate of 0.6125 litres/ha, indicate that site and consequent environmental variations may influence dissipation rates. At KOSFIP area, for rate of 0.6125 litres/ha, the residual levels of diazinon matching Codex MRL was achieved at 10.55 days which fitted the PHI at 12 days. While the suggested PHI for this low rate of application matches the label application conditions for rate 1.25

litres/ha and recommended PHI on *Brassica oleracea var. acephala*, low pest efficacy and subsequent impacts on the environment may result from this rate of application.

The RL₅₀ for the positive control rate of application, 1.25 litres/ha of diazinon in *Brassica oleracea var. acephala* reported 1.98 days. The RL₅₀ corresponded to 1.8 days in *Medicago sativa* (Talebi, 2006) and the mean RL₅₀ of 1.85 days for *Gossypium hirsutum L.* (Willis & McDowell, 1987). The values were however below the conventional RL₅₀ of 2.4 days for diazinon in leafy matrices but within the acceptable range of 0.8 – 5.2 days in plant matrices (Lewis *et al.*, 2016). This observation points out that the recommended rates of application of diazinon differ for each vegetable and the differences explains the variations in RL₅₀ values. The results compared with other studies done to validate diazinon PHI in various vegetables (Kabir *et al.*, 1970; Prodhon *et al.*, 2018). The latter literatures had concentrations of diazinon residues as 2.066 mg/Kg, 2.765 mg/Kg and 2.228 mg/Kg in eggplant, yardlong bean and brinjal, respectively, at day zero. The levels by the fifth day were 0.729 mg/Kg, 0.506 mg/Kg and 0.396 mg/Kg, respectively. Significant variations in magnitude were observed in residual levels on *Brassica oleracea var. acephala* for similar days post application but the trend was similar.

The levels for the current study were however lower than the findings by (Okworo, 2017), that reported 49.02 ± 0.26 mg/Kg on day zero followed with 24.64 ± 0.16 mg/Kg, 20.37 ± 0.14 mg/Kg, 10.47 ± 1.03 mg/Kg and 3.12 mg/Kg on day 2, day 4, day 7 and day 11, respectively. The RL₅₀ value for recommended rate was lower than RL₅₀ for half the recommended rate of application. It may be understood that optimum leaf distribution of the active ingredient, high efficacy on the target pests, and maximum growth dilution of the pesticide on the vegetable leaves resulted in higher rate of dissipation. Based on the RL₅₀ value for *Brassica oleracea var. acephala* at KOSFIP, the Codex MRL was achieved at 12.96 days post application. The corresponding Action PHI for this rate was 14 days. The APHI established corresponded to

recommendations by JMPR Appraisal report (FAO & WHO, 1993) and the findings of Okworo (2017). Given that the recommended rate of application must have met the optimum conditions for efficacy on target pest, the use of diazinon on *Brassica oleracea var. acephala* within the KOSFIP area of Kenya may be continued with a revised PHI of 14 days.

The RL₅₀ value of 2.42 days for rate of application of diazinon at 1.8625 litres/ha on *Brassica oleracea var. acephala* of KOSFIP corresponded to 2.6 days for *Poa annua* L (Sears & Chapman, 1979), maximum rate of application RL₅₀ values of 2.5 days for *Brassica oleracea alboglabra* cv. Guy Lon (Ripley *et al.*, 2003) and *Gossypium hirsutum* L. (Willis & McDowell, 1987). The resultant RL₅₀ values were within the standard RL₅₀ value for diazinon in plant matrices (Lewis *et al.*, 2016). To attain the Codex MRL of diazinon in *Brassica oleracea var. acephala*, a post application period of 16.53 days was required. Consequently, an Action PHI of 18 days was recommended for this rate of application. While the food safety level will have been achieved by the computed APHI, the 18 days period may be long enough to enhance pest resistance to diazinon. In addition, the long PHI will neither be environmentally sustainable nor economically viable and sustainable to the farmers.

At the double recommended rate of application, 2.5 litres/ha, RL₅₀ of 3.16 days was reported which corresponded to the mean RL₅₀ of 3.25 days in *Poa pratensis* L. (Sears *et al.*, 1987) and mean of 3.1 days in Turfgrass (Lemmon & Pylypiw, 1992). This 3.16 days' value was higher than the mean RL₅₀ of diazinon in plant matrices but was within the acceptable range of 0.8 – 5.2 days in plant matrices (Lewis *et al.*, 2016). Given that the two plant species reporting dissipation half-lives of 3.25 and 3.1 days were grasses that are treated with higher rates of diazinon (Diazol 60 EC Safety Data Sheet and Leaflet Front Label by Barmac, 30/11/2012), the finding confirmed that regardless of the plant matrix, higher rates of application require longer PHIs to attain the acceptable MRL. The computed post application period at which the

diazinon residues levels would match the recommended Codex MRL was 22.39 days equivalent to an Action PHI of 23 days.

These basic findings are consistent with research showing that post application period defined by label PHI is a significant good agricultural practice (GAP) that safeguards consumers of conventionally grown vegetables. The results suggest that for varying rates of application with resultant increased safe post application periods, there is need to propose Action Pre-Harvest Intervals based on the Codex MRL.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY

Levels of diazinon residues were detected and quantified in 92.5% of the farm-gate basket samples. Among the samples with detectable residual levels, 67.57% were above the Codex MRL while 32.43% were below the Codex MRL. About 40% of the samples presented residual diazinon quantities with health risk indices (HRI) > 1.0 for children and approximately 28% of the samples presented residual diazinon quantities that correspond to health risk indices (HRI > 1.00) for adults. Children were four times more at risk than the adults. When rates of diazinon application were varied with increasing active ingredient concentrations, the diazinon residues levels in *Brassica oleracea var. acephala* of KOSFIP were directly proportional to the rate of application of the pesticide. At the recommended PHI of 12 days, rates 0.6125 litres/ha, 1.25 litres/ha, 1.8625 litres/ha and 2.5 litres/ha, presented 0.0314 mg/Kg, 0.0700 mg/Kg, 0.1834 mg/Kg and 0.4862 mg/Kg, respectively. Similarly, for different rates of diazinon application, diazinon residues levels in *Brassica oleracea var. acephala* were inversely proportional to the post application periods. Rates 0.6125 litres/ha, 1.25 litres/ha, 1.8625 litres/ha and 2.5 litres/ha, required action PHIs of 12 days, 14 days, 18 days and 23 days, respectively, to realize the Codex MRL ≤ 0.05 mg/Kg of diazinon in the vegetable.

5.2 CONCLUSIONS

Most of the farm-gate *Brassica oleracea var. acephala* treated with diazinon within the KOSFIP area of Homa Bay County of Kenya had higher than recommended residue levels. The residual diazinon quantities may pose significant health risks to the consumers. The high levels suggested that most farmers of *Brassica oleracea var. acephala* who use diazinon within the KOSFIP area may not be observing good agricultural practices (GAPs). At the

recommended pre-harvest interval (12 days), rates ≥ 1.25 litres/ha yield diazinon residue levels $>$ Codex MRL (0.05 mg/Kg). since rates of application are subject to efficacy against pests, review of efficacy for rates , 1.25 litres/ha is necessary. While 1.25 liters/ha was the label recommended rate of application with a pre-harvest interval of 12 days, the study suggested a review of the PHI to 14 days. The recommendation was a result of diazinon residues levels in the *Brassica oleracea var. acephala* being above the Codex MRL on the 12th day post application. The study further suggested that alternative pesticides with shorter pre-harvest intervals may be used with strict observance of rates of application and pre-harvest intervals. This would mitigate against immediate health impacts on consumers of *Brassica oleracea var. acephala*.

5.3 RECOMMENDATIONS

5.3.1 Policy recommendations based on findings.

- a) There is need to restrict use of diazinon on *Brassica oleracea var. acephala* at KOSFIP and to consider alternative pesticides with shorter PHIs to reduce high levels of residual diazinon in farm gate vegetables.
- b) At the recommended PHI, an application rate of 1.25 litres/ha is inappropriate for *Brassica oleracea var. acephala* at KOSFIP.
- c) The recommended rate of 1.25 litres/ha application may be observed with a revised PHI of 14 days instead of 12 days.

5.3.2 Recommendations for further studies based on findings.

- a) There is need for a survey study into the extent of good agricultural practices (GAPs) with respect to pesticide use in the production of *Brassica oleracea var. acephala* and other vegetables within the KOSFIP area of Homa Bay County.

- b) Need for health risk index (HRI) determination for various age groups with estimated local weights, consumption rates and pesticide safety levels (PSL) adapted to the local environment.
- c) Comparative study on the effects of processing (washing, blanching, cooking) to establish if such processing methods significantly reduces diazinon residue levels in *Brassica oleracea var. acephala* of KOSFIP area
- d) Establish other agronomy-related variables that may influence residue levels in *Brassica oleracea var. acephala* and other vegetables produced in the study area.

5.4 Significance of the Study

This study has provided baseline information required for the establishment of Good Agricultural Practices (GAP) towards sustainable use of diazinon in production of *Brassica oleracea var. acephala* in the KOSFIP area. The findings may be used by agrochemical companies that manufacture and package diazinon to review label conditions and Safety Data Sheets (SDS) as used in production of *Brassica oleracea var. acephala*. The study has provided a basis for discouraging the use of diazinon in the production of *Brassica oleracea var. acephala* in KOSFIP and other environments. The policy makers and KOSFIP managers have been provided with appropriate data for the development of policies and training needs towards the realization of the Kenya vision 2030, the agenda 2063 of the African Union Commission and the sustainable development goals (SDGs) of the United Nations.

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APPENDICES

Appendix A: Types of pesticides

Classification by	Types of pesticides	Examples
Origin	Organic/Natural	Rotenone, Nicotine
	Synthetic	Diazinon
	Inorganic	Copper oxychloride
Mode of entry	Systemic	Thiamethoxam
	Contact	Lambda cyhalothrin
	Stomach poisons	Copper acetoarsenite
	Fumigants	Methyl bromide
Mode of action	Repellents	Cyfluthrin
	Physical poisons	Activated clay
	Protoplasmic poisons	Arsenic
	Respiratory poisons	Hydrogen cyanide
Stability	Nerve poisons	Malathion
	Chitin inhibition	Diflubenzuron
	Persistent	Endosulfan
Range of target organism	Degradable	Carbaryl
	Broad spectrum	Propineb/diazinon
Chemical structure	Specific/Selective	Primicarb
	Biopesticides	<i>Amblyseius cucumeris</i>
	Carbamates	Carbaryl
	Herbicides	Glyphosate IPA, Amaryl
	Organochlorines	DDT
	Organophosphates	Diazinon
	Pyrethroids	Lambda cyhalothrin
	Neonicotinides	Acetamiprid
	Avermectins	Abamectin, Selamectin
	Diamides	Flubendiamide
Toxicity (LD ₅₀ & LC ₅₀)	Triazines	Cyanazine
	Phenylpyrazoles	Fipronil
	Ia - Extremely hazardous	Parathion, Dieldrin
	Ib - Highly hazardous	Dichlorvos
	II - Moderately hazardous	Chlordane
	III - Slightly hazardous	Malathion
	U - Unlikely to present acute hazard	Difenoconazole

DDT: Dichlorodiphenyltrichloroethane

Source: (WHO, 2009; Yadav & Devi, 2017)

Appendix B: Uses and examples of pesticides based on target organisms

Category of Pest	Class of pesticide used	Examples
Acari	Acaricides	Abamectin
Algae	Algaecides	Bethoxazin
Aves/Birds	Avicides	Dimethoate
Bacteria	Bactericides	Bronopol
Fungi	Fungicides	Mancozeb
Herbs	Herbicides	Glyphosate IPA
Insects	Insecticides	Imidacloprid/ Diazinon
Lamprices	Lampricides	Chlorpyrifos,
Larvae	Larvicides	Ferric sodium EDTA
Molluscs	Molluscicides	Paradichlorobenzene
Moths	Moth Balls	Aldicarb
Nematodes	Nematicides	Hexaflumuron
Ovicia	Ovicides	Rotenone
Pisces	Piscicides	Picaridin
Rodents	Rodenticides	Bromodiolone
Silvicia	Silvicides	Cacodylic acid
Termites	Termiticides	Fipronil
Viruses	Virucides	Thymol
Others	Dessicants	Drione dust
	Repellents	Bifenthrin

(Atwood & Paisley-Jones, 2017; Pest Control Products Board, 2018; Vumilia et al., 2019; WHO, 2010; Yadav & Devi, 2017)

Appendix C: List of banned pesticides

Date	Common names of pesticides
1986	2,4,5-Trichlorophenoxybutyric acid, Chlordane, Chlordimeform, DDT, Dibromochloropropane, Endrin, Ethylene dibromide, Heptachlor, 5 Isomers of Hexachlorocyclohexane
1988	Ethyl parathion, Methyl parathion
1989	Captafol
2004	Aldrin, Benomyl, Carbofuran – Thiram combinations, Binapacryl, Chlorobenzilate, Dieldrin, Dinoseb Salts, Dinitro-ortho-cresol (DNOC) and its Salts of Ammonia, Potassium and Sodium, Ethylene Dichloride, Ethylene Oxide, Fluoroacetamide, Hexachlorobenzene, Mercury compounds, Pentachlorophenol, Phosphamidon
2009	Monocrotophos, all Tributyltin compounds
2011	Alachlor, Aldicarb, Endosulfan, Lindane
2019	Calcium cyanamide, Chlorophene/2-Benzyl, 4-Chlorophenol, Cypermethrin (Beta), Demeton, Demeton-O, Dinotefuran, Glufosinate (Including Glufosinate-Ammonium), Hydrogen cyanamide, Metaldehyde, Methoxyethyl mercury chloride (Memc), Prochloraz, Propiconazole, Propineb, Sodium dimethyl dithiocarbamate, Sulfluramid, Tepraloxym, Thiram, Ziram. In addition, 3 Chlorates are now listed separately instead of as one listing

DDT: Dichlorodiphenyltrichloroethane

Source: (Pest Control Products Board, 2018; Pesticide Action Network-International, 2019a)

Appendix D: Varieties of indigenous vegetables grown in different parts of the world

Region	Popular Vegetables
Europe	tomatoes, cucumbers, melons, onions, lettuce, spinach, cabbage, chicory, peas, aubergines, cauliflower, broccoli, kale and peppers
Americas	asparagus, kale, potatoes tomatoes, cucumbers, melons, onions, lettuce, spinach, cabbage, chicory, peas, aubergines, cauliflower, broccoli and peppers
Asia	beets, snow peas, chinese cabbage, eggplant, bean sprouts, white radish, squash, bamboo shoots, chinese clover, chinese artichoke, devil's bush, Japanes radish, asparagus, kale, potatoes, tomatoes, cucumbers, melons, onions, lettuce, spinach, cabbage, chicory, peas, aubergines, cauliflower, broccoli, peppers
Africa	African eggplant, African nightshade, leafy amaranth, bitter leaf, cow pea, marula, moringa, nettle, pigweed, teff, wild jute, wild mustard

Sources: (Abukutsa-Onyango, 2007, 2002; Akinola et al., 2020; Chweya, 1997; De Cicco, 2016; Hong & Gruda, 2020; Mnzava, 1997; Ojiewo et al., 2013).

Appendix E: Pesticide dissipation models and half-life functions for plant matrices

Pesticide dissipation model	Residual Concentration function	Dissipation half-life function
Zero order	$C_t = C_o - kt$	$t_{1/2} = C_o / 2k$
Half order	$C_t = (\sqrt{C_o} - kt/2)^2$	$t_{1/2} = \{(2 - \sqrt{2}) \times \sqrt{C_o}\} / k$
First order	$C_t = C_o e^{-kt}$	$t_{1/2} = \ln(2) / k$
One and half order	$C_t = ((1/\sqrt{C_o} + (kt)/2)^{-2})$	$t_{1/2} = (2\sqrt{2} - 2) / \sqrt{C_o}k$
Second order	$C_t = (C_o) / (1 + C_o kt)$	$t_{1/2} = 1 / (C_o k)$
Root function first order	$C_t = C_o e^{-k\sqrt{t}}$	$t_{1/2} = (\ln(2) / k) 2$
Root function (one-and-half order)	$C_t = (1/\sqrt{C_o} + (k\sqrt{t})/2)^{-2}$	$t_{1/2} = ((2\sqrt{2} - 2) / (\sqrt{C_o}k))^2$
Root function first order	$C_t = C_o / (1 + C_o k \sqrt{t})$	$t_{1/2} = (1 / C_o k)^2$
Root function second order	$C_t = C_o k \sqrt{t}$	$t_{1/2} = (1 / C_o k)^2$
Combined first + first order (biphasic)	$C_t = C_o e^{-k_1 t_1} + C_1 e^{-k_2 t_2}$	$t_{1/2} = \text{numerical least squares fitting required}$

C_o = residual pesticide concentration (mg/Kg) at time $t=0$; C_t = residual concentration (mg/Kg) at time t (days); C_1 = residual concentration at time $t > 0$; k, k_1, k_2 = pesticide dissipation rate constants (day⁻¹); $t_{1/2}$ = pesticide dissipation half-life. (Adapted from Fantke & Juraske, 2013).

Appendix F: Production trends and quantities of *Brassica oleracea var. acephala* in the counties of Kenya

County	Year 2015			Year 2016			%
	Area (Ha)	Volume	Value (KShs)	Area (Ha)	Volume	Value (Kshs)	
Kiambu	3,684	82,606	1,026,525,000	3,325	93,037	1,187,629,000	17.1
Nakuru	1,763	65,526	745,555,573	1,973	61,937	670,310,036	9.6
Kisii	1,821	24, 077	372,862,500	1,801	27,807	467,174,250	6.7
Bomet	871	19, 932	325,780,000	973	25,127	416,220,000	6.0
Narok	1,266	25, 883	433,817,379	1,835	21,027	394,819,300	5.7
Meru	1,397	25, 717	571,547,480	1,158	19,487	327,017,200	4.7
Nyandarua	1,685	27,357	277,713,623	1,380	22,025	239,096,350	3.4
Siaya	1,750	13,551	212,500,732	1,803	15,328	235,662,200	3.4
Homa Bay	957	8,543	237,741,000	806	8,426	225,645,000	3.2
Kericho	275	11,103	191,290,000	287	11,720	197,930,000	2.8
Nandi	840	12,740	110,120,000	1,070	16,531	186,550,000	2.7
Murang`a	594	6,681	121,308,000	579	9,584	184,137,750	2.6
Kakamega	1007	10,635	224,320,000	1,060	9,322	181,390,000	2.6
Elgeyo Marakwet	861	12,250	168,370,000	917	13,121	177,250,000	2.5
Taita Taveta	226	5,834	151,670,000	380	8,405	162,430,000	2.3
Bungoma	1,588	8,710	146,640,000	1,006	9,449	161,400,000	2.3
Lamu	276	5,533	176,598,000	291	5,420	140,520,000	2.0
Trans Nzoia	703	9,572	94,320,011	833	11,695	132,510,000	1.9
Nyamira	483	11,054	118,372,560	731	11,837	127,829,005	1.8
Others	8,842	95,185	1,671,650,353	10,141	76,837	1,139,262,680	16.4
Total	30,890	482,489	7,378,702,411	32,347	478,121	6,954,782,77	100

(Source: GoK, 2016)

Appendix G: Kenya's pesticide yearly imports in Kgs/Litres

SN	Pesticide category	2015 Imports	2016 Imports	% growth	2017 Imports	% growth	2018 Imports	% growth
1	Herbicides	2,025,803	4,139,478	104.34	4,354,232	5.19	5,899,020	35.48
2	Insecticides	1,889,135	2,751,206	45.63	3,746,427	36.17	4,536,117	21.08
3	Fungicides	2,476,783	4,965,268	100.47	3,673,816	(26.00)	5,163,972	40.56
4	Acaricides	577,800	1,372,254	137.50	1,598,260	16.47	280,978	(82.42)
5	Adjuvants	2,135,900	958,022	(55.15)	192,208	(79.94)	301,014	56.61
6	Miticides	218,876	128,414	(41.33)	124,557	(3.00)	252,628	102.82
7	Nematicides	111,348	280,966	152.33	75,051	(73.29)	319,814	326.13
8	Rodenticides	0	36,800	-	38,725	5.23	54,453	40.61
9	Others	559,729	52,626	(90.60)	933,364	1673.58	1,022,001	9.50
	TOTALS	9,995,374	14,685,034	353.19	14,736,640	1,554.41	17,829,997	550.37
	MEAN % GROWTH			44.15		172.71		61.15

Mean total volume imports for the 4 years: 14,411,761 Kgs/litres

Source: (Agrochemicals Association of Kenya, 2018). Figures in brackets are negative numbers indicating decline.

Appendix H_A: Pest Control Products Board approved label for diazinon (Diazol 60 EC)

Diazol 60EC
DIAZINON
INSECTICIDE

REGISTRATION NO. PCPB (CR) 0184
(Harbours ya wadudu)

GENERAL INFORMATION
DIAZOL is a non-systemic organophosphorus insecticide with acaricidal and nematocidal action effective against a wide range of insect pests on all major agricultural crops. It is a contact insecticide but it acts as a stomach poison against chewing insects. DIAZOL controls scale insects, mealy bugs, caterpillars, sucking bugs, aphids, thrips etc. On vegetables, fruits, trees and ornamentals.

RECOMMENDATIONS FOR USE
Timing of Application: Apply DIAZOL at a full cover rate 1.0-1.5L/ha. Spray at first sign of infestation. Repeat application after 10-14 days intervals. For scale insect control, an addition of medium light white oil at 1.2% of the total spray volume enhances control.
Preparation of the spray: Dilute recommended amount of DIAZOL with required amount of water in the spraying tank stirring well. Do not allow the spray mixture to stand. For high volume application, use 100-1000 L spray mixture.
For small holders' knapsack application, use 40-50ml/20L (30-37.5ml/15L). Use the provided measuring cup.
Note: On ornamentals, a trial application is recommended to check for plant tolerance of ornamental species and varieties prior to large-scale treatment.

Phytotoxicity: DIAZOL is well tolerated by crops when applied at the recommended rates of application.

Re-ENTRY INTERVAL (REI): 24 Hours after spray treatment
PRE-HARVEST INTERVAL
(Minimum days from the last application to harvest)

CROP	REI
Celery, Onion, Lettuce, Spinach, Brussel sprouts	10
Kales	12
Green beans, Cabbage, Cucumber, Coffee	7
Pepper, Cauliflower, Broccoli	14
Apples, Pears	5
Citrus	21

HAZARDS/PRECAUTIONS
This product is hazardous if swallowed, inhaled or if absorbed through the skin. Do not contaminate ponds or water ways by direct application; cleaning of equipment or disposal of waste.

MIALEZO KWA JUMILA
DIAZOL ni dawa aina ya organophosphorus iliyo na acaricidal/ Nematocidal. Inaambao husa aina nyingi za wadudu ambao wanashambulia mimea mhimili. Inaosa kwa kuuzwa, lakini pia ni sumu ya tumboi kwa wadudu wanaokula. Baschi ya wadudu wanaozuliwa na DIAZOL ni kama scales, kunguni, nonodo, aphids, thrips na wengine wanaoshambulia mboga, matunda, masaa na kahawa.

JINSI YA KUTUMIA
Wakati wa kutumia: Tumia DIAZOL kwa kiwango cha Lita 1.0-1.5 kwa hektari. Nyumiza dawa mara tu unapoonza wadudu. Rudia kutumia baada ya siku kumi hadi kumi na nne. Kwa kuziwa na kusangaziwa scales, ongeza asilimia moja hadi mbili ya mafuta meupe mepesi (white oil) kwa dawa.
Kutayarisha Dawa: Pima kiwango kinachopendekezwa cha DIAZOL na uchanganyie kikamilifu na kiasi cha maji kinachohitajika kwenye bomba la kutumia. Usiache mchanganyiko wa dawa ukaze kwa muda mrefu bila kutumia. Kwa wanunyuzizi wa kiwango cha jaa cha dawa, tumia lita mita moja hadi elfu moja ya mchanganyiko wa dawa ya kutumia kwa kila hektari.
Wakalima wa mashamba madogo wanaweza kuchanganya Lita 20 za maji na kiasi cha 40-50ml cha DIAZOL (30-37.5ml kwa Lita 15) wakitumia kipimo kilichoambatanishwa na dawa.
Fahamibu: Dawa inagotumiwa kwa kutumia masaa, ni sharti ijaribwe kabla sehemu ndogo kabla ya kutumia kwa kwenye sehemu kubwa ili kugundua ikiwa ina madhara yoyote kwa mimea.

MADHARA KWA NIMICA
DIAZOL haina madhara yoyote kwa mimea kitumia jinsi inavyo pendekezwa na kwa viwango vinyavyoruhusiwa.
MUDA WA KUSUBIRI KABLA KUNINGIA: Subiri masaa 24 kabla kuingia au kufanya kazi eneo lililokunyuziwa dawa.
MUDA KABLA YA KUVIWA
Kipindi cha kusubiri kabla ya kuvina

MUDA	MUDA
Vihanga, Spinach, Celery, Brussel Sprout, Lettuce	10
Sukuma Wiki	12
Maharagwe, Kabaji, Mbirimbi, Kahawa	7
Pilipili, Cauliflower, Broccoli	14
Apples, Pears	5
Michangwa	21

PEST CONTROL PRODUCTS BOARD
ACT 1982
VIKAZI WAY - Box 13754 NAIROBI

(Source: Pest Control Products Board Archives with permission on 29/05/2019)

Appendix H_B: Label with recommended application conditions and target pests

WHO CLASS II

DIAZOL[®] 60EC
INSECTICIDE DIAZINON

Guarantee: Diazinon 60% w/v
Formulation: Emulsifiable Concentrate
Classification: Organophosphate

General Description
DIAZOL 60 EC is a non-systemic insecticide for control of a wide range of insect pests including scale insects, mealy bugs, caterpillars, sucking bugs, aphids, thrips, caterpillars. It is used on vegetables, fruit trees, pineapples, ornamentals, coffee, cereals, sugarcane and tobacco.

Mode of Action
DIAZOL 60EC is a cholinesterase inhibitor. It acts with contact, stomach, and respiratory action.

Recommended Application Rates

Crop	Pests	Rates	Interval
Vegetables, e.g. French beans	Whiteflies, Thrips, Aphids, caterpillars	25ml/ 20 Lt 1.25Lts/ha	10 -14 days
Flowers e.g. roses	Thrips, caterpillars	1.25ml/Lt, 1.25Lts/ha	10 -14 days
	Aphids	1ml/Lt, 20ml/20Lt	10 -14 days
	Whiteflies	1.5ml/Lt, 30ml/20lt	10 -14 days

APPENDIX I: Krejcie and Morgan tables for the determination of sample sizes

TABLE 1
Table for Determining Sample Size from a Given Population

<i>N</i>	<i>S</i>	<i>N</i>	<i>S</i>	<i>N</i>	<i>S</i>
10	10	220	140	1200	291
15	14	230	144	1300	297
20	19	240	148	1400	302
25	24	250	152	1500	306
30	28	260	155	1600	310
35	32	270	159	1700	313
40	36	280	162	1800	317
45	40	290	165	1900	320
50	44	300	169	2000	322
55	48	320	175	2200	327
60	52	340	181	2400	331
65	56	360	186	2600	335
70	59	380	191	2800	338
75	63	400	196	3000	341
80	66	420	201	3500	346
85	70	440	205	4000	351
90	73	460	210	4500	354
95	76	480	214	5000	357
100	80	500	217	6000	361
110	86	550	226	7000	364
120	92	600	234	8000	367
130	97	650	242	9000	368
140	103	700	248	10000	370
150	108	750	254	15000	375
160	113	800	260	20000	377
170	118	850	265	30000	379
180	123	900	269	40000	380
190	127	950	274	50000	381
200	132	1000	278	75000	382
210	136	1100	285	100000	384

Note.—*N* is population size.
S is sample size.

Source: Krejcie & Morgan, 1970

APPENDIX J: Quality Control Parameters

- a) **Recovery units and corresponding Co-efficient of Variation (Precision), Repeatability and Linearity for six replicates of spikes for three concentrations of 10, 20 and 50 ppb.**

conc. (ppb)	Spiked Levels						C.V(%)	Repeatability	R ²	
	1	2	3	4	5	6				
50	a	44.851	41.830	44.258	49.934	52.687	41.883	9.685	4.4256	0.975
	b	43.656	53.799	44.175	54.652	47.712	47.700	9.616	4.5380	
	c	48.752	46.719	37.334	47.595	48.239	42.331	9.902	4.4384	
Mean	45.753	47.449	41.922	50.727	49.542	43.971	9.734	4.4670		
%	91.506	94.898	83.844	101.454	99.084	87.942				
20	a	-	21.153	19.287	20.102	17.545	18.402	(18.325)	(6.9145)	0.987
	b	19.461	17.864	20.071	18.454	19.337	18.968	4.109	1.8559	
	c	18.350	18.529	18.396	17.422	17.781	17.394	2.842	1.5003	
%	94.530	95.910	96.255	93.295	91.105	91.275				
10	a	12.078	11.442	11.364	11.084	11.277	13.379	7.280	1.9429	0.977
	b	12.942	12.835	13.209	13.799	11.836	12.522	5.127	1.7041	
	c	12.128	11.741	12.128	12.243	11.854	13.446	4.996	1.6424	
%	123.83	120.06	122.34	123.75	116.56	131.16				

- b) **Mean Recovery units for spikes used in the calibration curve of residual diazinon in *Brassica oleracea* var. *Acephala* of KOSFIP**

Spiked levels (µg/g)

10			20			50		
Rec.(%)	C.V(%)	Reptyy.	Rec.(%)	C.V(%)	Reptyy.	Rec.(%)	C.V(%)	Reptyy.
122.958	5.801	1.7631	93.728	8.426	3.4236	92.623	9.734	4.4673

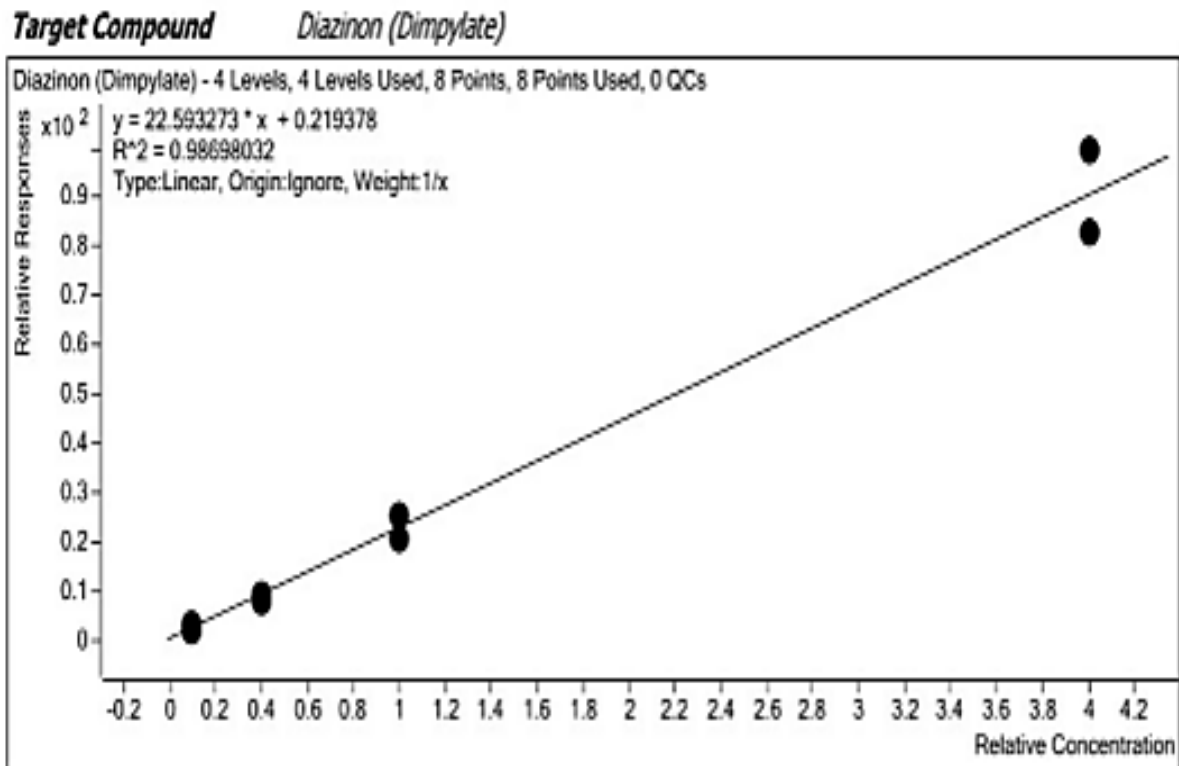
C.V – Coefficient of Variation; Rec. – Recovery; Reptyy. – Repeatability

- c) **Mean linearity, Limits of Detection (LODs), Limits of Quantification (LOQs) and Codex Maximum Residue Limits (MRLs) used in the calibration curve of diazinon residues in *Brassica oleracea* var. *acephala* of KOSFIP**

Linearity		Calibration Limits (mg/Kg)		MRLs(mg/Kg)	
R ²	Range(ppb)	LOD	LOQ	Kenya	EU
0.9797	5 - 200	0.01	0.03	0.05	0.01

EU – European Union; LOD – Limits of Detection; LOQ – Limits of Quantitation

Appendix K: Calibration curve for the quantitative determination of diazinon analyte in *Brassica oleracea var. acephala* samples



APPENDIX L: Optimization of LC-ESI-MS/MS Parameters for Quantitation and Confirmation

Cpd name	ISTD?	Precursor ion	MS1 resp.	Product ion	MS2 Resp.	Frag.(V)	CE (V)	Cell Ac(V)	Retention time (min)	Ref. window	Polarity
Diazinon (Dimpylate)	N	305.1	6490	169.1	6490	100	32	4	8.58	1.51	(+)
Diazinon (Dimpylate)	N	305.1	6490	97	6490	100	40	4	8.58	1.51	(+)
Dimethoate d6	Y	236	6490	205	6490	100	10	3	5.39	1.22	(+)
Dimethoate d6	Y	236	6490	131	6490	100	20	3	5.39	1.22	(+)
Malathion d10	Y	341	6490	132	6490	100	15	3	7.8	1.43	(+)
Malathion d10	Y	341	6490	100	6490	100	25	3	7.8	1.43	(+)

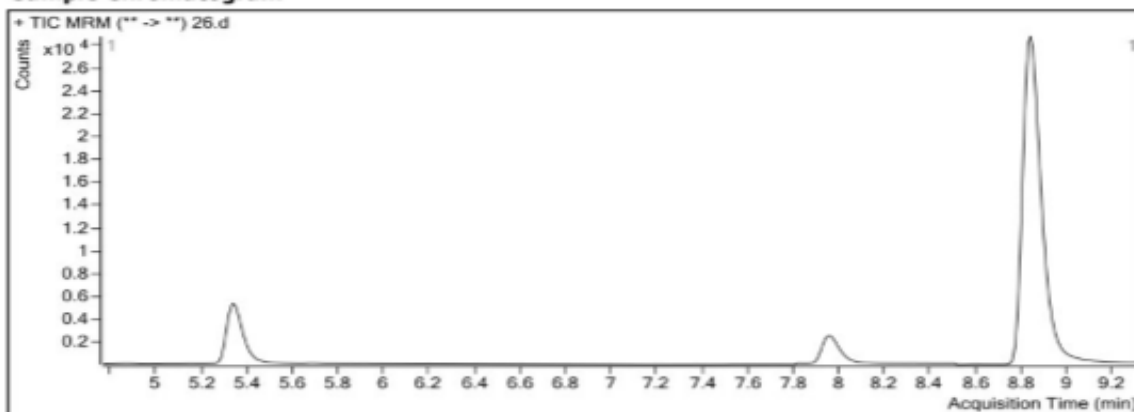
CE: - Collision Energy;

Appendix M: Selected sample chromatograms

Analysis Info

Acq Time	2020-03-03 21:14	Data File	26.d
Position	Vial 25	Sample Name	AA200478
Dilution	2	Sample Info	
Inj Vol	-1.00	Acq Method File	2308201901 George DIAZINON DMRM.m
Sample Type	Sample	Comment	

Sample Chromatogram



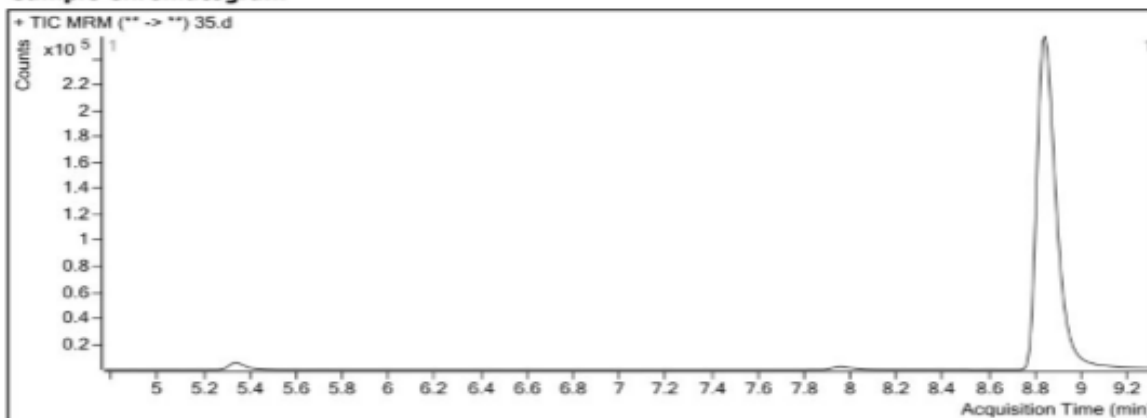
Quantitation Results

Compound	RT	Response	Conc
Malathion d10	7.959	7073	
Diazinon (Dimpylate)	8.844	101207	23.3071

Analysis Info

Acq Time	2020-03-03 23:18	Data File	35.d
Position	Vial 34	Sample Name	AA200487
Dilution	2	Sample Info	
Inj Vol	-1.00	Acq Method File	2308201901 George DIAZINON DMRM.m
Sample Type	Sample	Comment	

Sample Chromatogram



Quantitation Results

Compound	RT	Response	Conc
Malathion d10	7.959	7174	
Diazinon (Dimpylate)	8.844	912791	219.7634