KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

Determination of Pesticide Residues in Beans using AOAC QuEChERS Method and GC/MS

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AnMSc. THESIS SUBMITTEDAND PRESENTED

BY

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NIGERIA

July, 2021

DECLARATION

I hereby declare that this thesis titled (Determination of pesticide residues in beans using AOAC QuEChERS method and GC/MS) is a record of my research. It has neither been presented nor accepted in any previous application for higher degree.

AALIYAH OMOTAYO BELLO _____

Signature / Date

APPROVAL

This is to certify that this thesis by Aaliyah Omotayo Bellohas been read and approved as meeting the requirements of the Department of Chemical, Geological, and Physical Sciences (Chemistry Unit)for the award of the degree of Masters (M.Sc.) in Chemistry.

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DEDICATION

This project is dedicated to Almighty Allah (the giver and taker of life), the Most Beneficent, Most Merciful, the Master Planner that ever exist who made me pass through this school peacefully and my beloved Parents.

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TABLE OF CONTENTS

CHAPTER TWO

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES **Pages**

ABSTRACT

Pesticides used in agriculture for the control of various pests often leave residues in foodstuff. The misuse of pesticide for pest control in common among beans farmers. These has led to several rejection of Nigeria beans by the European Union members' country. Therefore, monitoring of pesticide residues in two varieties of beans from five markets randomly selected in Ilorin metropolis was carried out. Residue of dichlorvos and bifenthrin pesticides were determined in beans samples using AOAC modified QuEChERS methods followed by gas chromatography-mass spectrometry detection. The dichlorvos residues was found 100 % of the beans samples, which were found the range of 137.64 – 190.27 µg/kg and 137.00 – 163.10 µg/kg in brown and white beans sample respectively, while bifenthrin were detected in about 50 % of the samples, with concentration ranging from 3.84 -6.26 µg/kg and 5.24 – 7.62 µg/kg in white and brown beans respectively. The dichlorvos content was found to be higher than the maximum residue level, while bifenthrin was found lower than the maximum residue levels. The pesticide residues content was found to be higher in brown beans than white beans. Boiling and washing of the beans samples was found to reduce the pesticide residue content by 34.42 – 51.76 % in dichlorvos, and 11.45 – 28.65 % in bifenthrin. The concentration of dichlorvos in beans was high and could pose serious risk to human health. Therefore, there is need to educate and enlighten beans farmer on good agricultural practice in pesticide use.

CHAPTER ONE

1.0 INTRODUCTION

Food production capacity is faced with an ever-growing number of challenges, including a world population expected to grow to nearly 10 billion by 2050 and a falling ratio of arable land to population. Based on evidences, in 1900 there were 1.6 billion people on the planet; in 1992 this had risen to 5.25 billion and by the year 2050 it will reach 10 billion. World population is increasing by 97 million per year. This explosive increase in world population is mostly in developing countries and this is where the need for food is greatest and starvation threatens human life; as, FAO estimates that 690 million people are already undernourished(WHO, 2020). Civilization has been combating weeds, insects, diseases and other pests throughout history and there are many examples of how these pests have had a major impact on humans (Sarwar and Salman, 2016).

While the first recorded use of chemicals to control pests back to 2500 BC, it is really only in the last 50 years that chemical control has been widely used (Hock*et al.*, 1991). Many of the earliest pesticides were either inorganic products or derived from plants (i.e. burning sulphur to control insects and mites). It has been estimated that about 125,000 - 130,000 metric tons of pesticides are applied every year in Nigeria(FAO, 2013; Yusuf *et al*., 2019).

1.1 Pesticides

Pesticides are chemical substances that derive their name from the French word “Peste”, which means pest or plague and the Latin word “caedere”, to kill (Akunyili & Ivbijaro, 2006). The Food and Agricultural Organization (FAO), comprehensively defined pesticides (FAO, 2013), as “any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood

products or animal feedstuffs, or substanceswhich may be administered to animals for the control of insects, arachnids or other pests in or on their bodies".

The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. We use pesticides to cover a wide range of chemicals used to control insect pests, plant diseases, weeds, rats or other unwanted organisms. Currently, more than 800 pesticide active ingredients in a wide range of commercial products are registered for use in agriculture to meet food supply demands(FAO, 2013; Stoytcheva, 2011).

All pesticides are toxic to some plant or rodent species; at higher doses, they can also be toxic to farm animals, pets, and humans. In general, prominent insecticide families include organochlorines, organophosphates, and carbamates. Acute toxicity of insecticides for mammals' ranges from low to high. Herbicides used to control weeds have low acute toxicity for mammals; and fungicides are characterized as moderately toxic (Shokrzadeh & Saeedi, 2009).

1.2Benefit of Pesticide Use

A plentiful supply of fresh products is vital for a healthy population. Numerous scientific studies demonstrate the health benefits of regularly eating a variety of fresh fruit and vegetables (Abdulra'uf *et al*., 2016), and consumers are increasingly aware of these benefits. Agricultural productivity is a key to ensuring that this demand can be met at an affordable price; and crop protection products help increase productivity and usable crop yields. The crop protection industry's primary aim is to enable farmers to grow an abundant supply of food in a safe manner and prevent costs from increasing (Abdulra'uf *et al*., 2016). Food production

processes benefit from continual advancements in agricultural technologies and practices; in fact, a population now nearly twice as large has more food available per capita than 40 years ago. Tools such as herbicides, insecticides, and fungicides reduce crop losses both before and after harvest, and increase crop yields.Seyed *et al.*(2011), described the major benefits of pesticides and their role in food production as follows: Increase food quality and quantity, crop protection technologies allow producers to increase crop yields and efficiency of food production processes. Up to 40 percent of the world's potential crop production is already lost annually because of the effects of weeds, pests and diseases. These crop losses would be doubled if existing pesticide uses were abandoned. In addition, pesticides allow consumers to consume high-quality products that are free of insect blemishes and insect contamination. Crop protection chemicals that reduce, eliminate, and insect damage allow the consumers to purchase high-quality products free of insect fragments.

Decrease price of food: Because the use of pesticides improves crop yields, crop protection technologies also impact the cost of food. Without crop protection chemicals, food production would decline, many fruits and vegetables would be in short supply and prices would rise. Helping to keep food prices in check for the consumer is another large benefit of pesticides. Human health protection: Pesticides are the most effective substances to eliminate Insects that cause human diseases such as Malaria, Dengue fever, Lyme disease and West Nile virus loom large. Pesticides are used to protect human health against insect and fungi borne carcinogens, like aflatoxins, which is proceeding to hepatic and other cancers.

Therefore, pesticides must be used efficiently and effectively in order to strike a balance between their expected benefits and the possible risk to human health. This will enable their economic viability and environmental sustainability (Tadeo*et al*., 2012). Pesticides are widely used to control pests of fruits and vegetables, which are an important part of a healthy diet (Garrido-Frenich*et al*., 2012; Tuzimski, 2012). Use of pesticides in fruits and vegetables plays

a beneficial role in the provision of large quantities and high quality, low cost supply of fruits and vegetables (Bidari*et al*., 2011).

1.3Limitation of Pesticide Use

Food is the basic necessity of life and food contaminated with toxic pesticides is associated with severe effects on the human health. Hence it is pertinent to explore strategies that address this situation of food safety especially for the developing countries where pesticide contamination is widespread due to indiscriminate usage and a major part of population lives below poverty line. The four main groups of pesticides such as the organochlorine, organophosphate, carbamate, and pyrethroid insecticides (Ahmed *et al*., 2000; Smith &Gangolli, 2002) are of particular concern because of their toxicity and persistence in the environment; however, several of the banned pesticides are still used on a large scale in developing countries and continue to pose severe health and environmental problems. Pesticide use raises a number of environmental concerns, and human and animal health hazards.

Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil (Miller, 2004). Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination. As a result, we are closely exposed to pesticides in the food and water we consume and in the air we breathe. Unfortunately, these chemicals are non-biodegradable, persistent and get accumulated in the environment and thus into the human food chain. Despite regulatory measures, these compounds continue to be detected in measurable amounts in the ecosystem including marine life (Smith &Gangolli, 2002). In addition, pesticide use reduces biodiversity, reduces nitrogen fixation(Rockets, 2007), contributes to pollinator decline (Hackenberg, 2007;Haefeker, 2000;Wells, 2007; Zeissloff,, 2001), destroys habitat (especially for birds) (Palmer *et al*., 2007), and threatens endangered species (Miller, 2004).

It also happens that some of the pest adapt to the pesticide and don't die. What is called pesticide resistance, to eliminate the offspring of this pest, will be needed a new pesticide or an increase the dose of pesticide. This will cause a worsening of the ambient pollution problem. There is a growing concern that environmental chemicals, both natural and manmade, can cause:- Pesticide resistance in some pests; - Water, soil and air contamination that transfers the chemical residues along a food chain; Reduction of biodiversity and nitrogen fixation; Destruction of marine and birds' life and/or genetically defects in their next generations.

Changes in the natural biological balances, by means of reduction of beneficial and non-target organisms and insects, including predators and parasites of pests, and honeybees. On the other hand, the human population is exposed to these chemicals primarily through the consumption of pesticide contaminated farm products, leading to long term health hazards. Pesticides may induce oxidative stress leading to the generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzymes such as superoxide dismutase, catalase, glutothione peroxidase, glutathione reductase and glutathione transferase (Ahmed *et al*., 2000). Pesticide toxicity can result from ingestion, inhalation or dermal absorption. Also, many evidences show that pesticides are persistent in fish tissues, adipose tissue and other organs including brain cells, nervous system and endocrine glands, and even breast milk, etc., (Shokrzadeh*et al.,* 2009). Thus, continued exposure to these chemicals for a long period may result in various diseases listed below:

Neurological, psychological and behavioural dysfunctions, including Symptoms of mild cognitive dysfunction (leading to problems in identifying words, colours or numbers and inability to speak fluently), Parkinson's disease (PD) (Uversky*et al*., 2002; Xavier *et al*., 2004); Hormonal imbalances, leading to infertility, breast pain, menstrual disturbances, adrenal gland exhaustions and early menopause (Xavier *et al*., 2004). Immune system dysfunction, leading to immune suppression that cause potentially serious health risks in populations highly exposed

to infectious and parasitic diseases, and subject to malnutrition (Xavier *et al*., 2004). Reproductive system defects, including birth defects(Petrelli & Mantovani, 2002). Cancers, including brain cancers (i.e. neuroblastoma), soft tissue sarcomas (i.e. Ewig's sarcoma), and colorectal and testes carcinomas (Xavier *et al*., 2004).

Genotoxicity, including DNA damage in peripheral lymphocytes (Undeger and Basaran, 2002). Blood disorders, including leukaemia and non-Hodgkin's lymphoma (Zahm*et al*., 1997; Zahm& Ward, 1998), and abnormalities in liver and kidneys. Between specific age ranges, infants and children are at great risk from the effects of pesticides. Several studies suggest that children may be particularly sensitive to the carcinogenic effect of pesticides. There is a potential to prevent at least some childhood cancer by reducing or eliminating pesticide exposure (Zahmand Ward, 1998).

1.4Beans

A bean is a seed of one of several genera of the flowering plant family Fabaceae, which are used for human or animal food.The word bean and its Germanic cognates (e.g., German Bohne) have existed in common use in West Germanic languages since before the 12th century, referring to broad beans and other pod-borne seeds. This was long before the new World genus Phaseolus was known in Europe.*Phaseolus vulgaris*, also known as the common beans, green beans and French bean, among other names, is an herbaceous annual plant grown worldwide for its edible dry seeds or unripe. Seeds called "beans" are often included among the crops called "pulses" (legumes), although a narrower prescribed sense of pulses(Wikipedia, 2019).

Beans are high in protein, complex carbohydrates, folate, and iron. Beans also have significant amounts of fiber and soluble fiber, with one cup of cooked beans providing between nine and 13 grams of fiber. Soluble fiber can help lower blood cholesterol.

1.5Statement of the Problems

Pesticide use has been estimated to cause deaths of about 220 thousand people, with 3 million poisoning reported and 750 thousand cases of chronic illness, most especially in the developing countries (Atreya*et al*., 2011; WHO, 2006). Exposure of people to pesticides also reduces their productivity due to declining health condition, economic loss due to 10 payments for health services and change in social behavior due to loss of household income as a result of ill-health (McIntyre *et al*., 2006).

Beans is one of the most important inexpensive and popular vegetable crop in Nigeria. It is a rich source of essential vitamins which commonly grow during rainy season usually around the home stead by training its vine either on trees or by providing different kind of support. Despite being a prospective crop, high incidences of insect pest have limited the crop to its low yield and poor quality. In general, farmers in our country face significant yield loss of gains every year due to several attacks of various insect pest. Insect pests cause enormous quantity of yield loses in every season and every year. Although no regular statistical records are kept as per conservative estimative the yield loss in beans due to insect pest is reported to be about 12-30%. Five containers of beans exported from Nigeria to the Republic of Ireland was rejected and returned by the importers after the products were received with heaps of weevils. Currently, exportation of beans from Nigeria is banned by the European Union in June 2015, but it is not clear when the rejected product was exported on the ground that the produce contains high level of pesticide residues considered dangerous to human health(Nigerian Export Promotion Council, 2016).

1.6Justification of the Study

Pesticide residue are substances or mixtures of substances in food for man or animals resulting from the use of pesticide including any specified derivatives, such as degradation and conversion products, metabolites, reaction products and impurities considered to be of toxicological significance. Several reports show that global pesticide usage has increased

significantly during the last three decades' consequent with changes in farming practices and the increasing intensive agriculture. This extensive use of pesticides for agricultural and nonagricultural purposes has resulted in the presence of their residues in various environmental matrices, especially food stuff proving the high risk of these chemicals to human health and environment. Pesticide residues most commonly found in food samples of vegetal origin are pesticides that are intentionally applied to the plants to attack invertebrate pests (insecticides, acaricides, etc.) and plant diseases (fungicides). Every cultivated crop may be attracted by insects from the day it is planted until harvest. Hence, the value of chemical pesticides in controlling mosquitoes, termites, cockroaches, weevil and bowers, snails, rats and multitude of others animals have been great, and it is difficult to envisage modern disease control and agricultural programmes without some form of chemical control. If modern agriculture attempted to operate without chemical pesticides of some sort, production would probably decline precipitously in many areas, food prices would soar far higher and food shortages would become more severe.

1.7 Aim of the Study

The aim of the study is to determine the level of pesticide residues in two varieties of beans sold in Ilorin markets using AOAC modified QuEChERS extraction followed by Gas Chromatography Mass Spectrometry analysis.

1.8 Objectives of the Study

The objectives of the study are to:

1. investigate the efficiency of QuEChERS method for the extraction of pesticide residues in beans
2. determine the levels and distribution of bifentrhin and dichlorvos in beans in Ilorin metropolis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Properties of Pesticides

The physical and chemical properties of pesticides are influenced by the biotic and abiotic environmental processes, which therefore determines the persistency, mobility, transport and partitioning of pesticides. Pesticides resistance to photolysis, hydrolysis and

microbial degradation is determined by their chemical structure. The choice of extraction technique to be employed for their extraction in complex matrices is determined by the knowledge of their properties (Abdulra'uf *et al*., 2016).

2.1.1 Solubility

Solubility is a measure of the ability of a pesticide to dissolve in a solvent, which is usually water. Pesticides that are highly soluble in water dissolve easily. Such pesticides are more likely to move with water in surface runoff or to move through the soil in water than less-soluble pesticides. In the SDS, manufacturers use relative terms—such as miscible, dispersible, suspension, emulsifiable, and water solubility—to describe their product's solubility(Fishel, 2014). Some manufacturers will use a numerical value for this description, such as 2.9 mg/L or ppm. Pesticides with a value of 100 ppm or less are considered relatively insoluble, while pesticides with values greater than 1,000 ppm are considered very soluble(Zacharia, 2011).

2.1.2 Adsorption

Adsorption is the process whereby a pesticide binds to soil colloids, which are microscopic inorganic and organic particles in the soil. Colloid is derived from the Greek term meaning glue-like. These particles have an extremely large surface area in proportion to a given volume. It has been calculated that 1 cubic inch of colloidal clay may have 200–500 square feet of particle surface area. Adsorption occurs because of an attraction between the chemical and soil particles. Typically, oil-soluble pesticides are more attracted to clay particles and to organic matter in soil than water-soluble pesticides. Pesticide molecules with positive charges are more tightly adsorbed to negatively charged soil particles. A pesticide that adsorbs to soil particles is less likely to move from the application site than a chemical that does not adsorb tightly to the soil (Fishel, 2014).

2.1.3Reduction Reaction of Pesticides

Reduction of pesticides is a chemical reaction in which the substrate (pesticide) undergoes a reduction in oxidation state. The reducing agents in the environment are usually +H. For example, malathion undergoes a reduction reaction in acidic aquatic environment which proceed by the substitution of one of the ethyl group with +H resulting into the formation of two functional isomeric molecules of malathion monoacid at the end of one half life. However, malathiondiacid would be the product at extended reaction time (Kamal *et al*., 2008).

2.1.4Persistence

Persistence is the ability of a pesticide to remain present and active in its original form during an extended period before degrading. A chemical's persistence is described in terms of its half-life, which is a comparative measure of the time needed for the chemical to degrade. The longer a pesticide's half-life, the more persistent the pesticide. Persistent pesticide residues are sometimes desirable because they provide long-term pest control and reduce the need for repeated applications. However, some persistent pesticides applied to soil, plants, lumber, and other surfaces or spilled into water or on soil can later harm sensitive plants or animals, including humans. It is especially important to prevent persistent pesticides from moving off-site through improper handling, application, drift, leaching, or runoff (Nesheim and Fishel, 2009).

2.1.5Photodegradation of Pesticides

Photodegradation or photolysis is the breakdown or transformation of pesticides by sunlight that causes a rupture of chemical bonds. The organic molecule absorbs photons and become excited with the ensuing release of electron thus changing the molecule. Photolysis reactions are important for degrading organic molecules in the upper atmosphere, in shallow

aquatic environment, on foliage and on the surface of soils. Pyrethroids are particularly susceptible to photolysis reactions (Zacharia, 2011).

2.1.6Biodegradation

Biodegradation is the breakdown or transformation of pesticides by microbial agents which normally occurs in water and soil. The rate of microbial degradation depends highly on the amount and nature of pesticides present in the soil, the microbial population in the soil and soil conditions that favours microbial activities, such as warm temperature, favorable pH, adequate soil moisture, aeration and high organic matter content. The microorganisms participating in biodegradation include fungi, bacteria and other microorganisms that use pesticides as their substrate. Pyrethroids, organophosphates and some carbamates have been found to be more susceptible to biodegradation. However, most organochlorines have shown to be formidable to biodegradation due to the strength of C-Cl bond. The following is an example of microbial degradation of 2,4-D. The microbial degradation of 2,4-D can follow different pathways depending on the types of microbes present. Path "a" occurs when the bacteria Flavobacterina and Arthrobacter<u>sp</u> are present. Path "b" occurs when the fungus Aspergillus Niger is present (Linde, 1994).

2.1.7Volatility

Volatility is the tendency of a pesticide to turn into a gas or vapor. Some pesticides are more volatile than others. The likelihood of pesticide volatilization increases as temperatures and wind increase. Volatility is also more likely under conditions of low relative humidity.
The potential for a pesticide to volatilize is measured by its vapor pressure. This measurement may be described in units of Pa (Pascals) or mmHg (millimeters of mercury). Pesticides that have high vapor-pressure values are more volatile. Vapors from such pesticides can move off-

site and cause injury to susceptible plants. Some volatile pesticide products carry label statements that warn handlers of the product's potential for vapor movement (Fishel, 2014).

2.1.8Hydrolysis Reaction of Pesticides

Hydrolysis is a pH dependent reaction in which pesticides react with water (i.e. Hydrogen ion and hydroxy ion). Hydrolysis is one of the most common reactions that most pesticides undergo in the environment. Most organophosphates and carbamates have particularly shown to be highly responsive to hydrolysis reaction under alkaline condition. A pesticide that is very soluble in water will tend not to accumulate in soil or biota because of its stronger polar nature. This suggest that it will degrade via hydrolysis which is the reaction that is favoured in water(Zacharia, 2011).

2.2Classification of Pesticides

Pesticides can be classified by target organism, chemical structure, and physical state (Council on Scientific Affairs, American Medical Association, 1997). Pesticides can also be classed as inorganic, synthetic, or biologicals (biopesticides) which include microbial pesticides and biochemical pesticides. Plant-derived pesticides (botanicals), which have been developing quickly, include pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside(Kamrin, 1997).

2.2.1Classification Based on the Pest They Control

Herbicides used for killing weeds or herbs. e.g, Gramoxone; Insecticides- used for killing insects. e.g. Sevin; Fungicides-used for killing fungi. e.g. mankocide; Nematicides- used for killing nematode e.g. Furadan; Rodenticides- use for killing rodents (rat, mice) e.g. Klerat; Acaricides- used for killing aracnids (mites) e.g new mectin; Molluscocides-used for killing Molluscs (snails, slugs) e.g. Slugit (Abdulra'uf, 2014).

2.2.2Classification Based on Mode of Action: For Insecticides

Contact – kill only insects they are sprayed or dusted onto. Stomach acting- kill only insects that eat plant parts sprayed with insecticide e.g. Dipel. Systemic – This type of insecticide is transported within the plant and kill insects when they suck sap or eat parts of the plant. Fumigant- kill insects that inhale toxic vapours of the chemical e.g. phostoxin(Spark & Nauen, 2015).

2.2.3Classification Based onMode of Actionfor Fungicides

Contact (Protectant)- kill only fungi sprayed or dusted with the fungicide, or fungal spores, which come into contact with the fungicide. e.g. Kocide. Systemic (Eradicant)- This type of fungicides is transported within the plant and kills fungi growing within tissue of the plant.

2.2.4Classification Based onMode of Action for Herbicides

Pre-emergence – an herbicide applied to the soil during the period after planting and before germination (usually 1-5 days after land preparation). e.gGesaprim. Selective – an herbicide that kills small weeds and seeds but leave the crop unharmed e.gGesagard. N.B selective herbicides are specific for certain crops; Post-emergence – an herbicide applied to growing weeds after crop emergence or transplanting e.g. Fusilade, Gramoxone, Round-up; Contact- an herbicide that kill only soft green parts of weeds sprayed with the herbicide e.g. Gramoxone; Systemic- an herbicide that is absorbed into the plant after spraying, and is transported to other plant parts where it causes death e.g Round-up; Non-selective- an herbicide that kills all plants sprayed. e.g Round-up.

2.2.5Classification Based on Formulation

Dust- pesticide prepared as dry fine particles e.g. Sevin 85 WP; Granules (G)- Pesticides prepared as large dry particles e.g Furadan; Wettable Powders (WP)- consist of finely divided particles with other substances that enable the powder to be mixed with water to form a stable suspension e.g. kocide; Emulsifiable concentrate (EC)- a pesticide dissolved in an organic solvent to which an emulsifier is added to enable proper mixing; Dry Bait-pesticide mixed with edible products to form dry pellets, which are attractive to pests. e.gKlerat; Smokes-the pesticide is mixed with an oxidant and combustible material, which generates hot gas e.g. mosquito coil.

2.2.6Classification Based on Chemical Structure

2.2.6.1Organophosphorus Pesticides (OPPs)

Organophosphorus pesticides are esters and organic acid halides of phosphoric and phosphonicacids, with all the H atoms replaced by organic moieties and are the most widely used pesticides. Their structure is made up of a central phosphorous atom bonded to several side chains. They include organophosphate, organophosphonates, organophosphinates, organophosphoramidates, organophosphorothioates, organophosphorodithioates, organophosphonodithioates, organophosphonothioates, and organophosphoroamidothioates depending on the substituent atoms (Chambers *et al.*, 2010; Kamrin, 2000; Thompson &Richardson, 2004). They are highly toxic and were by-products of chemical warfare research in the development of nerves gas agent during World War II, such as sarin, somanand tabun (Abdulra'uf*et al.*, 2016).

OPPs vary in the groups attached to the central phosphorus atom through the sigma bonds, such as OR, SR, CR and NR in a variety of combinations.Organophosphates (phosphorus acid derivatives) are compounds in which the phosphorus atom is surrounded by

four oxygen atoms, while in phophonates (phosphonic acid derivatives) contains three oxygen atoms and one carbon atom surrounding the phosphorus atom and phophinate has two oxygen atom and two carbon atoms bonded to the central phosphorous atoms. One or more of the oxygen atoms attached to the central phosphorous atom could be replaced by sulfur and or nitrogen (Abdulra'uf, 2014).

OPPs are synthesized by the reaction of elemental phosphorous with sulphur to produce P2S5 or by direct chlorination to yield PCl5. The P2S5 and PCl5 produced by these reactions are then converted to several intermediates through which most OPPs are synthesized. OPPs are stable in cool, dry and anhydrous conditions, but can be altered when exposed to light, heat and/or water and may undergoes hydrolysis, oxidation and rearrangement reactions (Chambers *et al*., 2010).

2.2.6.1.1 Toxicology and Mode of Action of OPPs

OPPs are generally acutely toxic and they poison insects and mammals. Their toxicity depends on the nature of the leaving group attached to the phosphorus atom. The most toxic OPPs have oral LD50 in the range of 1–30 mg/kg, while the moderately toxic group has LD50 between 30–50 mg/kg and the less toxic group has LD50 between 60 and 1300 mg/kg. Their mode of action is through the irreversible inhibition of acetylcholinesterase enzyme which causes interference in the nerve endings of the central nervous system. The leaving group is displaced by nucleophilic attack of the active site of serine when OPP phosphorylates the acetylcholinesterase enzyme (AChE). OPPs with P S undergo oxidative desulphuration by phosphorylating a hydroxyl group on serine in the active site of the enzyme to their

corresponding and highly polarized P O analogues. The reaction which results in the formation of a transient intermediate complex is partially hydrolyzed with the loss of the leaving group, resulting in the formation of a stable and largely unreactive enzyme. The reaction can be carried out by chemical, biological and/or environmental agents and it speeds up the breakdown of acetylcholine produced in the nerve endings. Acute poisoning could cause respiratory failure, cardiac arrest which could result in death (Waxman, 1998; Kamrin, 2000; Ecobichon, 2001; Thompson and Richardson, 2004; Costa, 2008; Yu, 2008).

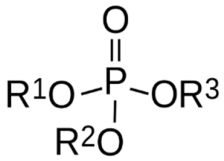


Fig 2.1: General Structure of OPPs (Chambers *et al*., 2010)

2.2.6.2Carbamate Pesticides (CPs)

Carbamate pesticides (CPs) are esters of carbamic acids in which the 3 replaceable H atoms (1 attached to C and 2 attached to N) of carbamic acid are displaced by aliphatic, aromatic or heterocyclic radicals to become carbamate pesticides (Yu, 2008). They are analogs of the drug physostigmine, a methyl carbamate alkaloid extracted from the plant Physostigmavenenosum, called Calabar bean, which grows naturally in West Africa (Kamrin, 2000; Ecobichon, 2001). They are colourless, odourless crystalline compounds, which are relatively stable to air, light and heat during storage. They are non-persistent environmental pollutants and are more selective with less toxicity on mammals. They have several of chemical structures, which are all derivatives of carbamic acid and can be divided into three subclasses (Costa, 2008). They include Methyl carbamates with aromatic radicals (e.g. carbaryl); Methyl carbamates and dimethylcarbamates with heterocyclic radicals (e.g. carbofuran); Methyl carbamates of oximes with a linear structure (e.g. aldicarb).

2.2.6.2.1 Toxicology and Mode of Action of CPs

CPs have different degrees of toxicities, ranging from moderate, to high and extremely high toxicity and are open to different biotransformation reactions which are enzyme catalyzed, in which the reaction stages involve hydrolysis and oxidation (Costa, 2008). They have broad spectrum of biological activity and relatively short half-life (Ni *et al.*, 2005). They inhibit acetylcholinesterase by a reversible carbamylation of the serine hydroxyl group in the active site of the acetylcholine (neurotransmitter) at the parasympathetic neuroeffector junction, leading to the persistent amount of acetylcholine on the cholinergic postsynaptic receptor (Kamrin, 2000; Knaak, *et al*., 2008; Zhang,*et al*., 2010).

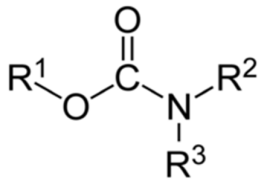


Fig. 2.2: General Structure of Carbamate Pesticides (Kamrin, 2000).

where R_1 is an alcohol, oxime, phenyl ring or heterocyclic group, R_2 and R_3 are either hydrogen or a methyl group. The carbamates in which the 2 H-atoms attached to the nitrogen are replaced have been found to be less toxic. Thus, in the manufacturing of the methyl carbamates, the second H-atom is not replaced because the monoalkyl substituted is more toxic than the N-disubstituted compounds (Yu, 2008).

2.2.6.3 Organochlorine Pesticides (OCPs)

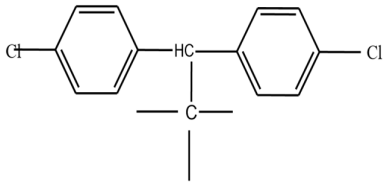
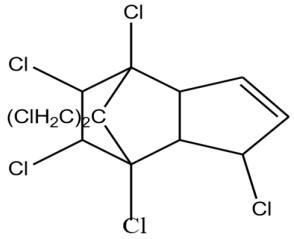
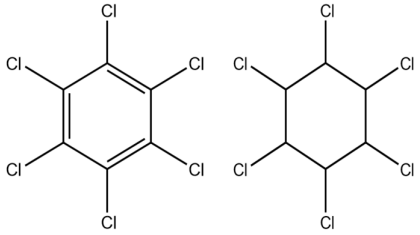
The organochlorine pesticides are hydrocarbon compounds which contain carbon, chlorine and hydrogen atoms with diverse group of agents. They include the chlorinated ethane derivatives and their analogues, though lacking a common structure are characterized by one or more chlorine atoms. OCPs are divided into three distinct chemical classes:

dicholorodiphenylethanes, cyclodienes and chlorinated benzenes and cyclohexanes and related caged structures as shown in Table 2.1 below. Members of each group share similar or identical composition, but may have different stereo-structures and shapes and also differ in toxicities. OCPs also include fabricated chemicals such as polychlorinated biphenyls, dioxin and dibenzofurans which are by-products of several industrial processes. Their different chemical structures and properties lead to their broad range of uses. Most of the OCPs have been banned for use in some countries, but are still in use in the developing countries because of the effectiveness and low cost (Abdulra'uf*et al.,* 2013).

2.2.6.3.1 Toxicology and Mode of Action of OCPs

OCPs are stimulants of the nervous system which are absorbed orally, by inhalation and by dermal exposure. After exposure to the OCPs, some of the absorbed doses are stored in the fat tissues as an unaltered parent compounds. They interfere with fluxes of the cations in the nervous system and affect the nerve fibers along the length of the fiber. They increase neuronal irritability by disturbing the transmission of nerve impulse and disrupt sodium/potassium balance surrounding the nerve fibers. They are also known or suspected to be endocrine disrupting compounds, which interfere with the anabolic and catabolic activities of the natural hormones, responsible for the maintenance of homeostatics, reproductive development and behaviour. Acute exposure to a high dose of OCPs have been found to cause motor unrest, spontaneous and uncontrolled movement of the body and hypersensitivity to external stimuli, but are rapidly reversible when the concentration falls below some threshold levels which varies depending on the structure of the OCPs (Ecobichon, 2001; Costa, 2008).

Table 2.1: Structural Classification of Organochlorine Pesticides

Dichlorodiphenylethanes		DDT Dicofol Perthane Methoxychlor Methlochlor
Cyclodienes		Aldrin Dieldrin Heptachlor Chlordane Endosulfan
Chlorinated benzenes Cyclohexanes		HCB, HCH, Lindane (α-BHC)

(Abdulra'uf, 2014).

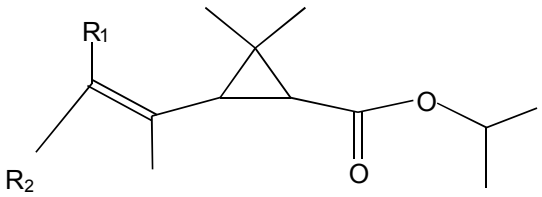
2.2.6.4 Pyrethroid Pesticides (PPs)

Pyrethroid pesticides (PPs) are synthetic materials which originate from the naturally occurring pyrethrins. Pyrethrins are extracts of the dried heads of flowers of *Chrysanthemum cinerariaefolium*, which has about 50% of active insecticidal ingredients. Natural pyrethrins consist of six ketoalcoholic esters of chrysanthemic and pyrethric acids, namely pyrethrins I & II, jasmolin I & II and cinerin I &II (Costa, 2008; Kaneko, 2010 Chambers *et al.*, 2010). The instability of the natural pyrtethrins in daylight led to the development of pyrethroid, the synthetic analogs (Costa, 2008). PPs are classified into types I and II compounds. The type I pyrethroid pesticides are produced by esters lacking α-cyano substituent and are made up of esters of chrysanthemic acid and alcohols with a furan ring and terminal side chain substituent

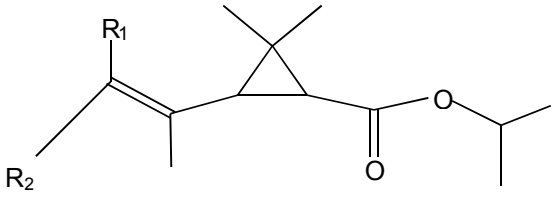
and is unstable in the presence of light, air and elevated temperatures. Type II are made up of 3-phenoxybenzyl alcohol derivatives in the alcohol substituent, with the α-cyano substituent, and they are stable to light, air and temperature, with high insecticidal activities (Abdulra'uf*et al.,* 2016).

2.2.6.4.1 Toxicology and Mode of Action of PPs

Pyrethroid pesticides causes dermal and respiratory allergies, and have similar mode of action in insects and mammals, but mammals are relatively resistant due to their faster metabolic activities, higher body temperature and lower sensitivity of the target sites. PPs interfere with the balance of sodium ions in the nerve ending, disrupt the voltage-gated sodium channels, by binding to the α-subunit of the sodium channel thereby slowing down the rate of activation and inactivation of the channel (i.e. causing delay in the closing of the sodium channel) and rendering it hypersensitive (Waxman, 1998; Kamrin, 2000; Ray, 2004;Costa, 2008). Acute toxicity of PPs consists of two types. Type I syndrome includes sudden change in behavior, startle response and body tremor, and is produced by PPs with their esters lacking α-cyano substituents, while type II PPs with their esters consisting of the α-cyano substituents, induces slow depolarization of the nerve membrane and reduces the action potential amplitude and causes intense salivation, coarse tremor which can lead to chronic seizure (Yu, 2008).



A



B

Fig. 2.3: General Structures of (A) Type I and (B) Type II Pyrethroids(Abdulra'uf, 2014).

2.3 PesticidesSelected for this Study

For the purpose of this research work, bifenthrin and dichlorvos pesticides were selected. The selected pesticides are widely used by farmers on fruits and vegetables.

2.3.1 Bifenthrin

Bifenthrin is an insecticide and a member of the pyrethroid family of chemchemicals. It is considered a Type I, non-cyanopytheroid with isomeric enrichment. The stereoisomers 1S, 3S and 1R, 3R are found in commercial products(Fecko, 1999).

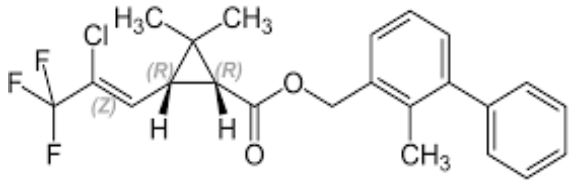


Fig. 2.4: Molecular structure of Bifenthrin (Amweg *et al*., 2005)**.**

The technical International Union of Pure and Applied Chemistry (IUPAC) name for bifenthrin is 2-methylbiphenyl-3-ylmethyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-l-enyl)- 2,2-dimethylcyclopropanecarboxylate and the Chemical Abstracts Service (CAS) registry number is 82657-4-3.Bifenthrin was first registered for use by the United States Environmental Protection Agency (U.S. EPA) in 1988.

2.3.1.1Physical/Chemical Properties:

Bifenthrin is pale tan to off-white in color. It can be crystalline, waxy solid or viscous liquid. It has a weak, aromatic odor.Vapor pressure: 1.81 x 10^{-7} mmHg at 25 °C; Octanol-Water Partition Coefficient(K_{ow}): 1.0 x 10^{6}'; Henry's constant: 7.2 x 10^{-3} atm·m^3/mol; Molecular weight: 422.9 g/mol; Solubility (water): <1 μg/L; Soil Sorption Coefficient (K_{oc}): 1.31 x 10^5 - 3.02 x 10^5.

2.3.1.2 Mode of Action

Target Organisms

Bifenthrin is designed to be effective by contact or ingestion. Bifenthrin is a Type I pyrethroid that affects the central and peripheral nervous system by interfering with sodium channel gating. Pyrethroids delay the closure of the sodium channel. Type I pyrethroids such as bifenthrin tend to hold the channel open for shorter times compared to type II pyrethroids

Non-target Organisms

The mechanism of action of pyrethroids, including bifenthrin, is the same for mammals and invertebrates.Pyrethroids are less toxic to mammals compared to insects because of mammals' higher body temperature, larger body size, and lower sensitivity of the ion channel sites (Peterson *et al*., 2006).

2.3.1.3 Uses of Bifenthrin

Products containing bifenthrin are used on cereals, cotton, corn, alfalfa, hay, grass seed, some fruits, ornamentals, and vegetables.Uses for individual bifenthrin products vary widely. Producs containing bifenthrin are used against a wide range of insects and mites(Amweg *et al*., 2005).

2.3.2Dichlorvos

Dichlorvos is an insecticide that is a dense colorless liquid. It has a sweetish smell and readily mixes with water. Dichlorvos used in pest control is diluted with other chemicals and

used as a spray. It can also be incorporated into plastic that slowly releases the chemical. Dichlorvos is used for insect control in food storage areas, green houses, and barns, and control of insects on livestock. It is not generally used on outdoor crops. Dichlorvos is sometimes used for insect control in workplaces and in the home. Veterinarians use it to control parasites on pets(Mennear, 1998).

Toxic by inhalation, skin absorption or ingestion and used as a pesticide. May be found in the form of a dry mixture where the liquid is absorbed onto a dry carrier. Dichlorvos is an alkenyl phosphate that is the 2,2-dichloroethenyl ester fo dimethylphosphate. It has a role as an acetylcholinesterase inhibitor, an anthelminthic drug, a cholinesterase inhibitor, an antibacterial agent and an antifungal agent (Okoroiwu and Iwara, 2018)

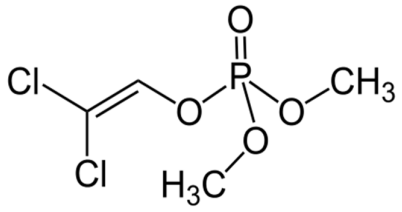


Fig 2.5: Molecular structure of dichlorvos (Riar, 2014)

2.3.2.1Mode of Action

Dichlorvos like other organophosphate insecticides, acts on acetylcholinesterase, associated with the nervous systems of insects. Evidence for other modes of action, applicable to higher animals, have been presented.It is claimed to damage DNA of insects (Pancetti *et al*, 2007).

2.3.2.2Uses of Dichlorvos

Dichlorvos is effective against mushroom flies, aphids, spidermites, caterpillars, thrips, and whiteflies in greenhouses and in outdoor crops. It is also used in the milling and grain handling industries and to treat a variety of parasiticworm infections in animals and humans. It

is fed to livestock to control botflylarvae in manure. It acts against insects as both a contact and a stomachpoison. It is available as an aerosol and soluble concentrate. It the most commonly used organophosphate pesticide in developing countries (Binukumar *et al*., 2010).

2.4 Extraction Techniques for the Analysis of Pesticide Residues in Food Samples

Analysis of pesticide residues and other contaminants in food samples is becoming increasingly important due to the health hazards caused by their accumulation in human tissue. The purpose of any analytical study is to obtain information about substances and analytes present in the sample. Analytical process involves several steps: sampling, sample preparation, separation, quantification and data analysis. Sample preparation is a very important step and indeed the bottleneck of analytical methodologies, in the analysis of fruits and vegetables for the presence of pesticide residues in fruit and vegetable samples. The first step in any instrumental analysis is sample preparation, which involves the isolation or extraction of the desired analytes from the sample matrix, since they are present at trace concentration (usually µg.kg or less). This helps in the elimination of any interferences and also reduces the volume of extracts, thereby concentrating the analytes (Abdulra'uf*et al*., 2013).

The type, nature and composition of sample and the nature and concentration of analytes to be isolated or extracted determines the choice of separation and detection method to be used, and this also dictates the type of sample preparation to be employed, since the efficiency of any analysis is determined by the sample preparation step. The current trend of microextraction techniques is aimed at a reliable and accurate analysis of contaminants from complex samples. It is focused on the reduction of sample and solvent volume, with the automation/coupling of the sampling step to the analytical instruments, while maintaining the high throughput performance, low cost operation and improvement of the sample preparation, such as extraction, concentration, isolation of analytes, and clean-up.

The conventional solvent-based sample preparation methods: liquid-liquid extraction (LLE), solid phase extraction (SPE), accelerated solvent extraction (ASE), matrix solid phase dispersion (MSPD), usually require various matrix pretreatment steps, which are tedious, time consuming, contains multistep techniques, require large volumes of sampleand toxic solvents which impose environmental pollution and health hazards with high operation cost. Therefore, it is necessary to reduce the number of sample preparationsteps in order to reduce the sources of error. Microextraction techniques are recently developed sample preparation methods which are vital as well as effective and efficient ways to save time, reduce solvent use and operation cost, and can efficiently measure the trace analytes embedded with high molecular mass compounds in complex sample matrices (Abdulra'uf *et al*., 2016).

2.4.1Microextraction Techniques

The microextraction techniques have been developed by different researchers, to corroborate the recent advances in the development of highly sensitive and efficient analytical instrumentation. Instrumental techniques like gas and liquid chromatography and capillary electrophoresis which are compatible with the microextraction technique, and coupled to different detectors (mass spectrometry, diode array detector, ultraviolet detector,etc), have been developed for qualitative and quantitative analysis of pesticide residues and other contaminants from foods. Prior sample preparation is necessary in order to extract, isolate and concentrate the analytes of interest from the complex fruits and vegetables matrices, which contain high molecular mass compounds. The low cost and ease of hyphenation of these microextraction techniques to analytical instruments helps to reduce errors, due to contamination and sample losses. In this review, recent advances in different microextraction techniques used in the analysis of pesticide residues from food samples are discussed, with a major focus on their methodologies, advantages, limitations and future trends (Abdulra'uf*et al*.,2013).

2.4.1.1Solid Phase Microextraction(SPME)

SPME saves preparation time and reduces the overall cost of analysis. Its development has addressed the need for rapid sample preparation (Risticevic *et al*., 2009). It offers the benefits of short sample preparation time, solvent-free extraction, small sample volumes, and analyte concentration from solid, liquid, or gaseous samples. It has helped to address some of the drawbacks of conventional sample preparation methods and can easily be automated, resulting in high-throughput analysis(Risticevic*et al*., 2009). Due to its remarkable analytical characteristics including linearity, reproducibility, repeatability, and low LOD, SPME is an efficient and effective extraction technique in the analysis of pesticide residues in fruits and vegetables (Abdulrauf *et al*., 2012).

SPME is a very attractive alternative technique in sample preparation that results in high selectivity, sensitivity, and versatility with minimum matrix interferences(de Fatima, 2000; Fytianos, 2007). It has improved detection limits (Pawliszyn, 1997), and wide applications for the analysis of volatile and semi volatile organic compounds when combined with GC/MS, and for thermally labile, polar and nonvolatile compounds(Aulakh, *et al*., 2005; Lambropoulou*et al*., 2007), when coupled to liquid chromatography(Falqui-Cao, et al., 2001), and capillaryelectrophoresis(Rodriguez, *et al.*, 2003). SPME uses a chemically inert fused-silica optical fiber or metal alloys coated on the outside with a thin film of sorbent(Arthur*et al*., 1992) as the extraction stationary phase, containing a polymeric organic compound, e.gcarbowax (CW), divinylbenzene (DVB), polydimethylsiloxane (PDMS), polyacrylate (PA), or carboxen (CAR), or a mixture of polymers(Kataoka,2000; Pawliszyn,1997;Simplicio, 1999), that are permanently attached to a stainless steel rod. The fiber holder consists of a spring-loaded plunger, a stainless barrel, and an adjustable depth gauge with a hollow septum-piercing needle (Kataoka, *et al*., 2000) housed in a modified syringe(Beltran, 2003; Chai *et al*., 2008).SPME is based on analyte partition and establishment of equilibrium between the analyte in the sample and the stationary phase of the coated fused silica, which can either be liquid or

solid particles suspended in liquid polymer or a combination of both(Arthur*et al*., 1992; Aulakh*et al.*, 2005; Lambropoulou*et al*., 2007). The attainment of equilibrium depends on the partition coefficient (Aulakh*et al*., 2005), which reflects the chemical composition of the extraction phase and, hence, its selectivity towards a given analyte. The partition coefficient is expressed as the ratio of the concentration of the analyst in the stationary phase to its concentrationin the sample.

There are two distinct steps in SPME;

1. Partitioning of the analytes between the extracting stationary phase and the sample matrix in the direct immersion (DI) mode and the partitioning among the fiber, headspace (HS), and the sample matrix in the HS mode,and,

2.The desorption of the concentrated extract(Risticevic *et al*., 2009), thermally when coupled to GC or with a mobile phase solvent when coupled to LC.\

2.4.1.2 Stir Bar Sorptive Extraction(SBSE)

Stir bar sorptive extraction (SBSE), as a microextraction method, was first introduced by Baltussen *et al*. (1999). This technique has gained wide acceptance as a highly efficient sample preparation method for enrichment of solutes from aqueous samples. The extraction is controlled by the solutes partitioning coefficient between the polymer coating and the sample matrix and by the phase ratio between the polymer coating and the sample volume. For a polydimethylsiloxane coating and aqueous samples, this partitioning coefficient resembles the octanol-water partitioning coefficient.In comparison to solid phase micro-extraction, a larger amount of sorptive extraction phase is used, and consequently, very high sensitivities in environmental, food and biomedical fields trace analysis. SPME and SBSE have been compared for the analysis of different compounds, as organochlorine pesticides and organophosphorus insecticides.

SBSE is a microextraction technique similar to SPME but with a greater extraction capacity. It helps to overcome the small volume of the coated SPME fibers for a better enrichment factor and it delivers better sorptive-phase mass and higher surface area as a result of larger volume of the PDMS (Baltussen *et al*., 2002;Beceiro González *et al*., 2012;Ridgway *et al*., 2007). In the SBSE technique, a 10 to 40 mm long magnetic stir bar coated with thick layer (about 50–300 µL) of polydimethylsiloxane(PDMS) liquid phase as the extracting phase (Baltussen *et al*., 1999; Kataoka, 2010; Tankiewicz *et al*., 2011).

The mechanisms of SBE are similar to those of SPME but differ in the design of extraction system, with SBSE having higher enrichment factor, which is determined by the amount of extractive phase. The extracted analyte are adsorbed on the PDMS coated rod, by stirringthe sample solution with the rod for a given time. The rod is removed from the sample and the adsorbed analyte can be desorbed thermally into GC system, which provides high chromatographic resolution and better sensitivity or by means of liquid solvent into LC system for improved and better selectivity (Baltussen *et al*., 2002; Hyötyläinen& Riekkola, 2008; Kawaguchi *et al.*, 2006; Prieto*et al*., 2010).

The major limitation of SBSE technique is the polarity of PDMS (non-polar liquid), which implies that it is best used for low polar analytes as the recovery will be low for highly polar analytes (Kawaguchi *et al*., 2006; Sánchez-Rojas*et al*., 2009), which is as a result of weak hydrophobic interactions, longer desorption time, due to the large volume of the PDMS, and it also requires reconstitution of the extracted analytes on the stir bar, since it cannot be injected directly into the split/splitless injector port of the GC (Hyötyläinenand Riekkola, 2008;Nogueira, 2012; Prieto *et al*., 2010). The tedious reconstitution step can lead to loss of analytes and introduction of contaminants, but this has been eliminated by the use of thermal desorption unit (TDU) online to GC system (Blasco*et al*., 2002).

All the studies reached the same conclusion, whereby the SBSE concentration capability was better than those presented by SPME because of polydimethylsiloxane (PDMS) film phase that covers the bar is thicker. In the SBSE technique, the sample is stirred with a glass enclosed magnetic stir bar coated with a layer of PDMS, resulting in a distribution of analytes between the aqueous sample matrix and the PDMS layer. The glass jacket is useful to immobilize sorbents onto the stir bar but it may lead to low stability and reproducibility because the glass jacket is relatively fragile.

According to the work of Nurul Auni*et al*, (2018), they introduced a glass jacket-free SBSE configuration by introducing a low cost hollow glass cylinder as a template for coating the SBSE polymeric phase. This template is important for reproducing the same bar characteristics from batch to batch. Thus, the work aims to develop the prepared homemade glass jacket-free SBSE using PDMS sorbent for the determination of the selected triazine herbicides at trace levels from water samples prior to their determination by gas chromatography-flame ionization detector (GC-FID). An experimental design was used to investigate the influence of the principal parameters affecting glass jacket-free SBSE extraction of triazines. Thus, for determining the best experimental conditions, a central composite design (CCD) was applied. The developed method was then tested using different quality control approaches including detection limit and recovery assay.

2.4.1.3 QuEChERS

The QuEChERS sample preparation method has become the most popular technique to prepare fruits, vegetables and other commodity sample for multi residue pesticide analysis.QuEChERSmethod allow users to quickly and easily extract their compounds of interest from a sample and prepare them for further analysis.The two most common methods are the European Committee for Standardization[CEN] method EN15662 (CEN, 2008) and AOAC method (AOAC, 2007)

2.4.1.3.1QuEChERSTheory and Methodology

Anastassiades*et al.*(2006), described the QuEChERS method involves two main steps: a liquid-liquid extraction and a dispersive solid phase extraction clean up step. There are three commonly used methods that have given rise to all of the current QuEChERS methods: the original method, the AOAC 2007.01 method, and finally the European Standard EN 15662 method, the latter two employing the use of buffers and various salts in the method. It is obvious that the QuEChERS method used will depend upon the analytes of interest; however, each method would require optimization of items such as the solvent system, the amounts and types of salts present, and the choice of d-SPE sorbent(Anastassiades*et al.,* 2003).

The basic steps for each method are the same, a LLE between an organic phase and water with the use of salts for liquid-liquid partitioning. The sample is shaken then centrifuged and an aliquot of the organic extract is removed and subjected to a d-SPE clean up using magnesium sulfate ($MgSO_4$) and a sorbent that will bind matrix interferences such as primary secondary amine (PSA). The sample can then be analyzed using GC or LC(Anastassiades*et al.,* 2003).

During the LLE, acetonitrile, ethyl acetate, or acetone are the three organic solvents most commonly used during QuEChERS, as they are safer than chlorinated solvents. ACN is the most frequently used organic solvent of the three as it minimizes the amount of interferences extracted while also extracting a broad range of analytes. The LLE also includes the use of salts to drive the analyte of interest into the organic solvent as well as aid in phase separation. Sodium chloride (NaCl) and magnesium sulfate ($MgSO_4$) are used in the original and European methods, with the latter also using citrate buffering salts including sodium citrate dibasic sesquihydrate ($Na_2HCitr\text{-}1.5H_2O$) and sodium citrate tribasic dehydrate ($Na_3Citr\text{-}2H_2O$)(Anastassiades*et al.,* 2003). Salts aid in the partitioning of polar compounds by increasing the ionic strength that can result in salting out or salting in depending on the

properties of the compound and solvents. Adding salt can increase the polarity of a solvent, thus increasing the solubility of the polar compounds in that solvent.(Lehotay *et al.,* 2005) The d-SPE step is similar in all three methods: a clean-up sorbent such as primary secondary amine (PSA) removes polar matrix interferences such as sugars, fatty and organic acids, and some pigments, and $MgSO_4$ is added to act as a desiccate, removing any water transferred with the organic phase (Anastassiades *et al.*, 2006). An aliquot of the liquid is then transferred to a vial for analysis. In the European method it is suggested to acidify the extract with formic acid once QuEChERS is complete in order to improve the storage conditions for base-sensitive pesticides. The sorbent chosen can be optimized based upon the analytes of interest to provide the cleanest sample. Each sorbent removes specific interferences to provide a cleaner sample depending on the composition of the matrix(CEN, 2008).

Some examples include: primary secondary amine (PSA) which decreases levels of organic acids and lipids based upon weak ion exchange, end capped where residual silanols of the sorbent have been reacted with reagent so they are no longer active which removes lipids and non-polar interferences, graphitized carbon black (GCB) which works to bind planar analytes and lower the recovery of pigments, and aminopropyl which is similar to PSA but has less effect on base sensitive analytes, providing higher recovery of those analytes. Dual phase sorbents that combine two or more of the aforementioned sorbents are also of interest, especially for specifying the removal of certain compounds. For example, a dual phase sorbent of GCB/PSA removes pigments while helping to retain planar analytes whereas GCB alone would remove the latter. The choice of sorbent would depend on the matrix of the sample as well as the composition of the analytes of interest. The optimized result would provide high recovery and remove matrix interference peaks(CEN, 2008).

For an extraction, it is desired that the distribution constant be greater than one so as to have a majority of the analyte in the extraction solvent. The extraction is also effected by type

of solvent, temperature, and pH used during the process. The pH of the aqueous sample phase must be adjusted to provide the non-ionized form of the analyte of interest in order to maximize extraction (Schmidt, 2015). The temperature of the system is related to thermodynamics in that it affects the spontaneity of the process.

All of these factors play a role in the extraction process and must be considered here. This will be accomplished through optimization of the various parameters as stated above. By evaluating the solvent type, pH, temperature, and agitation of the system, the results will provide information on the interactions occurring as well as the thermodynamic and kinetic properties of the system (Anastassiades, 2006).

Fig 2.6: Pictorial Representation of the QuEChERS steps (Agilent-Technologies, 2010).

2.4.1.3.2Applications of QuEChERS in Food Analysis

Agricultural products can have quite complex matrices and thus the extraction of analytes from this matrix is well suited to QuEChERS. There has been a multitude of research performed on the use of QuEChERS for the extraction of pesticides, fungicides, insecticides, and herbicides for various matrices(Martinez-Dominguez *et al.,* 2014). A brief summary will be discussed in order to expose the reader to this area of QuEChERS, followed by a more in depth analysis of other applications of QuEChERS including both GC and LC analyses.

Dasika *et al*., (2012), looked at the extraction of pesticides in fruits and vegetables using both GC and LC/MS and found that, in general, the three methods provided similar results for all matrices resulting in an overall recovery of 98% and residual standard deviations less than 10%. It was found that the original unbuffered method did provide slightly lower recoveries for pH dependent pesticides with the acetate buffering AOAC 2007.01 method providing the most consistent and highest recovery for these compounds including pymetrozine and thiabendazole.

According to Yang*et al*., (2012),QuEChERS was the most efficient extraction, providing recoveries of 27.3-120.9%. The effect of washing and cooking foods was evaluated for the removal of pesticides. During the study, 31 foods and 44 pesticides were monitored using QuEChERS and LC-MS/MS before washing, after washing, and after processing such as boiling. It was concluded that levels of the pesticides did decrease significantly or were eliminated after washing and cooking with the exception of green chilies. The level of acetamiprid actually increased during boiling and stir-frying of green chilies.

Herbicide, fungicide, and insecticide residue analysis in food products or soil is also commonly performed using QuEChERS. Various forms of the QuEChERS method have been investigated for the extraction of herbicides in polished rice, yogurt, milk (Li, *et al*., 2013).Baby food was analyzed for 10 fungicides using QuEChERS and LC-ion trap-MS/MS. The analysis of real samples showed the presence of some of the fungicides investigated, but at a level below 10μg/kg, the cutoff level designated by the European Commission Directives (Gilbert-Lopez*et al*, 2012). The concentration level of insecticide was evaluated in banana leaves used to feed cattle and hogs and was performed using QuEChERS and GC-MS/MS with 89-104% recoveries and less than 9.1% RSD (Gonzalez-Curbelo *et al*, 2013).

2.5Instrumental Methods of Analysis

2.5.1Gas Chromatography

Gas chromatography is a common type of chromatography used inanalytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture(Pavia *et al*, 2006).

A gas chromatograph uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase(Linde, 2012). As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to

alter the order or time of retention are the carrier gas flow rate, column length and the temperature (Linde, 2012).

2.5.1.1Principle of Gas Chromatography

The principle in gas chromatography involves separation of volatile components of the sample based on their partition co-efficient. This is ratio of solubility of substance in between gaseous mobile phase and stationary liquid phase. The components of the sample that are partitioned into gas come out first while others come later. Gas chromatography runs on the principle of partition chromatography for separation of components. The stationary phase is a liquid layer supported over a stationary phase while the mobile phase is an inert and stable gas (Raja & Barron, 2021)

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase material (Harris, 1999). Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column(Linde, 2012).

2.5.2Liquid Chromatography

Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase, the different solutes will interact with the other phase to differing degrees due to differences in adsorption, ion-exchange, partitioning, or size. These differences allow the mixture components to be separated from each other by using these differences to determine the transit time of the solutes through a column (Brian, 2000).

2.5.2.1 Principle of Liquid Chromatography

Brian (2000), advanced that simple liquid chromatography consists of a column with a fritted bottom that holds a stationary phase in equilibrium with a solvent. Typical stationary phases (and their interactions with the solutes) are: solids (adsorption), ionic groups on a resin (ion-exchange), liquids on an inert solid support (partitioning), and porous inert particles (size-exclusion).

Mayure (2017), classified liquid chromatography as follows:thin layer chromatography (TLC), paper chromatography (PC), ion exchange chromatography (IEC), column chromatography (CC).

2.5.3Thin Layer Chromatography

In thin layer chromatography,the stationary adsorbents are applied to a planar glass or plastic surface and the mobile phase (sample to be analyzed) is allowed to flow over the stationary phase. For separation of components in the sample anyone of the following techniques is used: Adsorption, Ion exchange, Partition,Gel filtration(Mayure, 2017).

2.5.4Column Chromatography:

a) **Liquid/ liquid chromatography**

In this method, the liquid stationary phase is retained on the surface of packing by physical adsorption and the sample (mobile phase) is allowed to percolates through the column (stationary phase)(Mayure, 2017).

b**) Liquid/ solid (Adsorption) chromatography**

In this method, the stationary phase is formed by a solid adsorbent, generally silica and alumina in powdered form. The sample solution is allowed to percolate through the stationary phase in the column (Mayure, 2017).

c)**Gel permeation chromatography**

In this method, the separation is based on molecular size and shape. The gel permeation column is packed with a stationary phase in the form of a gel contacting pores of a specific size. The sample solution is allowed to flow over the column bed then the sample penetrates the pores in the packing gel (depending upon the size and shape of the molecules). The large molecules don't penetrate the gel and leave the column earlier(Mayure, 2017).

2.5.5Ion Exchange Chromatography

In ion exchange chromatography, the exchange of ions between solution and solid insoluble in contact with solution takes place. The ion exchange process is reversible. In this process, when a sample is introduced at the top of the ion exchange column, the sample ions are displaced into solution again and then re-exchange on to the resin. This process continuous till the sample ions leave the column. Now the various sample ions corresponding to the different components in the sample are hold on to the resin to different extent. This cause the different time of passage through the column for different ions and separation of the sample components is achieved(Mayure, 2017).

2.6Detection Methods

A detector is a device used in gas chromatography (GC) or liquid chromatography (LC) to detect components of the mixture being eluted off the chromatography column. There are two general types of detectors: destructive and non-destructive. The destructive detectors perform continuous transformation of the column effluent (burning, evaporation or mixing with reagents) with subsequent measurement of some physical property of the resulting material (plasma, aerosol or reaction mixture). The non-destructive detectors are directly measuring some property of the column eluent (for example UV absorption) and thus affords greater analyte recovery(Wikipedia, 2020).

2.6.1 Gas Chromatography (GC)Detectors

As solutes elute from the column, they interact with the detector. The detector converts this interaction into an electronic signal that is sent to the data system. The magnitude of the signal is plotted versus time (from the time of injection) and a chromatogram is generated.Some detectors respond to any solute eluting from the column while others respond only to solutes with specific structures, functional groups or atoms. Detectors that exhibit enhanced response to specific types of solutes are called selective detectors (Raja & Barron, 2021).

Most detectors require one or more gases to function properly. There iscombustion, reagent, auxiliary and makeup gases. In some cases, one gas may serve multiple purposes. The type of detector gas is dependent on the specific detector and is fairly universal between GC manufacturers. The flow rates for each type of detector varies between GC manufacturers. It is important to follow the recommended flow rates to obtain the optimal sensitivity, selectivity and linear range for a detector (Qian & Peterson, 2017).

2.6.1.1 Flame Ionization Detector (FID)

Flame ionization detector is a scientific instrument that measures analytes in gas stream. It is frequently used as a detector in gas chromatography. The measurement of ion per unit time make this a mass sensitive instrument (Skoog *et al*., 2017). Standalone FIDs can also be used in applications such as landfill gas monitoring, fugitive emissions monitoring and internal combustion engine emissions measurementin stationary or probable instruments(McWilliam and Dewar, 1958). FID measurements are often labelled "total hydrocarbons" (ASTM, 2015)or "total hydrocarbon content" (THC), although a more accurate name would be "total volatile hydrocarbon content" (TVHC), as hydrocarbons which have condensed out are not detected, even though they are important for e.g safety when handling compressed oxygen(Dauenhauer, 2015).

2.6.1.2 Nitrogen Phosphorus Detector (NPD)

The nitrogen phosphorus detector (NPD) is also known as thermonic specific detector (TSD) is a detector commonly used with gas chromatography, in which thermal energy is used to ionize an analyte (Wolfgang, 2001). It is a type of flame thermonic detector (FTD), the other being the alkali flame –ionization detector (AFID also known as AFD). Withnthis method, nitrogen and phosphorus can be selectively detected with a sensitivity that is 10^4 times greater than that for carbon(Burgett*et al.,* 1977).

2.6.1.3 Electron Capture Detector (ECD)

Electron capture detector is a device for detecting atoms and molecules in a gas through the attachment of electrons via electron capture ionization. The device was invented in 1957 by James Lovelock and is used in gas chromatography to detect trace amounts of chemical compounds in a sample (Lovelock, 1958). The ECD is used for detecting electron-absorbing components (high electronegativity) such as halogenated compounds in the output stream of a

gas chromatograph. The most sensitive detector known. Allows for the detection of organic molecules containing halogen, nitro groups(Lovelock,1974).

2.6.1.4 Thermal Conductivity Detector (TCD)

The thermal conductivity detector (TCD), also known as a katharometer, is a bulk property detector and a chemical specific detector commonly used in gas chromatography (Sunil *et al*., 2018). This detector senses changes in the thermal conductivity of the column eluent and compares it to a reference flow of carrier gas. Since most compounds have a thermal conductivity much less than that of the common carrier gases of helium or hydrogen, when an analyte elutes from the column the effluent thermal conductivity is reduced, and a detectable signal is produced(Budiman and Zuas, 2015).

2.6.1.5 Flame Photometric Detector (FPD)

Flame photometric detector uses a photomultiplier tube to detect spectral lines of the compounds as they are burned in a flame. Compounds eluting off the column are carried into a hydrogen fueled flame which excites specific elements in the molecules, and the excited elements (P, S, Halogens, some metals) emit light of specific characteristics wavelengths(Higson, 2004). The emitted light is filtered and detected by a photomultiplier tube(Harris, 2016; Higson, 2004). In particular, phosphorus emission is around 510-536 nm and sulfur emission is around 394nm(Higson, 2004; Harris, 2016). With an atomic emission detector (AED), a sample eluting from a column enters a chamber which is energized by microwaves that induce a plasma(Higson, 2004). Theplasma causes the analyte sample to decompose and certain elements generate an atomic emission spectra (Higson, 2004). The atomic emission spectra is diffracted by a diffraction grating and detected by a series of photomultiplier tubes or photo diodes(Higson, 2004).

2.6.1.6Photo-Ionization Detector (PID)

A Photo ionization detector (PID) uses an ultraviolet (UV) light source to ionize chemicals to positive and negative ions that can be easily counted with a detector. Ionization occurs when a molecule absorbs the light energy. The gas becomes electrically charged. These charged particles produce a current that is then amplified and displayed on the meter as "ppm". The first commercial application of PID was in 1973 as a hand-held instrument for the purpose of detecting leaks of VOCs, specifically vinyl chloride monomer (VCM), at a chemical manufacturing facility. The PID was applied to gas chromatography three years later, in 1976 (Driscoll & Clarici, 1976).

2.6.1.7Electrolytic Conductivity Detector (ELCD)

The electrolytic conductivity detector is a destructive, mass-sensitive selective detector. Its main use is for regulated methods designed for selective detection of halogen-containing compounds(Klee, 2012). Electrolytic conductivity detector is a detector highly sensitive and selective to chlorinated compounds. Chlorinated compounds eluting from the column are pyrolized in aflow of hydrogen inside a nickel tube furnace at 850^0C and produce hydrochloric acid. The acid is transported into an electrolytic conductivity is proportional to the number of pyrolized molecules. Compounds containing organic nitrogen and sulfur will undergo the same reaction to produce ammonia and hydrogen sulfide, however, this type of interference is rare in VOC analysis of environmental matrices (Popek, 2003). This detector can be used for halogens, nitrogen and sulfur but not for all three simultaneously. The principle of operation of the ELCD is extremely simple. The gases eluting from the column are passed with hydrogen through a small nickel furnace heated to about 850-1000^0C. Under these conditions halogen compounds are reduced to HX, nitrogen compounds to NH_3 and sulfur compounds to H_2S. These gases are all very soluble in water and if they are passed into a circulating water stream the electrical conductivity will show major changes as the gases emerge (Adlard, 2003).

2.6.1.8Mass Spectrometer (MS)

A mass spectrometer is composed of three main components: An ion source, a mass analyser, and a detector. In the ion source, the sample is impacted with a beam of electrons (70eV) obtained from a tungsten filaments, exciting and ionizing the analyte molecule, causing fragmentation derived from the analyte's structure. The ion source used here was electron ionization, which is a form of hard ionization meaning it produces more fragmentation than a softer techniques such as chemical ionization(McNair & Miller, 1998).

In the mass analyzer, the ions that were created in the ion source are separated based on their mass-to-charge (m/z) ratio in quadrupole mass analyzer and by their kinetic energy in a time of flight mass analyzer. A quadrupole is composed of four parallel rods at right angle to each other with alternating electrostatic charges and a magnetic field formed by a radio frequency surrounding the pole. The ion travel through the center of the poles, reaching a detector. The entire range of masses can be scanned or selected number can be analyzed using selected ion monitoring (SIM). SIM confirms an analyte identity (Barnes, 2012; Grob & Barry, 2004).

2.6.2 Liquid Chromatography Detectors

Detectors for LC are designed to take advantage of some physical or chemical attribute of either the solute or mobile phase in the chromatographic process in one of four ways:A bulk property or differential measurement, Analyte specific properties, Mobile phase modification, Hyphenated Techniques (Swartz, 2005).

2.6.2.1 UV-Visible Detectors

An Ultraviolet detector (also known as UV detector or UV-Vis detector) is a type of non-destructive chromatography detector which measures the amount of ultraviolet or visible light absorbed by components of the mixture being eluted off the chromatography columnThe UV-visible absorbance detector is the most common LC detector in use today since many compounds of interest absorb in the UV (or visible) region (from 190–600nm)(Meyer, 2010). There are three different types of UV detectors: fixed wavelength detectors that rely on distinct wavelengths, and variable and photodiode array detectors (DAD or PDA) that rely on one or more wavelengths generated from a broad spectrum lamp(Dong and Wysocki, 2019).. Fixed wavelength detectors, the backbone of early LC systems, are cheap and simple, but are in limited use today. The most common fixed wavelength detectors use the 254nm output from a low pressure mercury lamp, the reason many variable wavelength and photodiode array applications today still use this wavelength out of sheer habit(Dong & Wysocki, 2019).

2.6.2.2 Fluorescence Detectors

Fluorescence detectors (FL) measure the optical emission of light by solute molecules after they have been excited at a higher energy wavelength and can be very sensitive for compounds that have native fluorescence or that can be made to fluoresce through derivatization(Swartz, 2010a).FL detectors can be as much as 100 times more sensitive than a UV detector, making them particularly useful for trace analyses, or in sample limited or low concentration sample situations (Ewa & Anna, 2017).

2.6.2.3 Electrochemical Detectors

For compounds that can be oxidized or reduced the electrochemical (EC) detector is one of the most sensitive and selective LC detectors available.They are termed as “Electrochemical detectors” for the reason being that they usually measure the current associated with the oxidation or electron of solutes. They act as amperometer or coulometer in

HPLC (Willard and Dean, 1986). They are classified as equilibrium and dynamic detectors. The suitability of these detectors depends on the volumetric characteristics of the solute molecules in the aqueous or organic mobile phase. They are sensitive to changes in the flow rate or composition of the eluent and require working electrode, reference electrode and auxiliary electrode(Ismail*et al*., 2005).

2.2.2.4 Chiral Detectors

Chiral detector continuously measures the optical angle of rotation of the effluent. It is used only when chiral compounds are being analyzed.Many compounds, particularly drugs, exist in enantiomeric forms (Hutt and Tan, 1996)that can possess significantly different pharmacological properties, and chromatographic separation of enantiomers can be complimented by the use of detectors capable of responding to the different chiral forms(Ariens, 1984).

Chiral detectors in flow cell form essentially mimic their bench top counterparts; polarimeters (PL), optical rotary dispersion (ORD), and circular dichroism detectors (CD)(Jasco-Inc., 2003). Polarimeters measure the degree of rotation of polarized light as it passes through the sample. The amount of rotation is dependant upon both the concentration and molecular structure of the analyte(Swartz, 2010b). ORD detectors operate quite similar to polarimeters but at lower wavelengths. CD detectors measure the difference in absorption of right and left circularly polarized light as an analyte flows through the detector cell(Swartz, 2010a).

2.2.2.5Refractive Index Detection

They are also one of the bulk property detectors and are based on the change of the refractive index of the eluent from the column with respect to pure mobile phase(Raymond, 1995). There are different types of Refractive index detectors: Christiansen effect detector,

interferometer detector, thermal lens detector and the dielectric constant detector. They are mostly used for detection of non-ionic compounds that neither fluoresce nor absorb in the UV region. They face the drawback of being less sensitive, need of temperature control and less suitability to gradient elution(Kenmore & Ersline, 1997).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Apparatus and Glassware

The glassware used in this study include: Polypropylene tube(5 and 20ml), blender, centrifuge, conical flask, measuring cylinder(10ml and 100ml), beaker(250ml), volumetric flask(50ml and 100ml), reagent bottle, fluttered filter paper, 45polypropylene syringe filter(Sigma Aldrich) and sample bottle. The following apparatus were used for sample processing; Food processor,Weighing balance, Centrifuge.

All glassware including the glass vials were cleaned thoroughly with detergent and bristle brush, then rinsed with deionised water and dried in the oven.

3.2 Analytical Reagents and Standards

Neat pesticide standard ofbifenthrin and dichlorvos (100 mg) was purchased from Sigma Aldrich. All solvents (methanol, acetonitrile, acetone carbon tetrachloromethane, chlorogorm, chlorobenzene) used were pesticide grade, and were purchased from Sigma Aldrich, USA. Sodium chloride was purchased from Merck.

3.3 Experimental Procedures

3.3.1 Standard Stock Solution

A working standard mixture containing 10µg/mL of the pesticide (bifenthrin and dichlorvos) were prepared daily in acetone from the stock pesticide standard (100µg/mL), by mixing aliquot of the standard and kept in the freezer at 4 °C before use. Aliquot of 50µL of the working standard solution was used to spike 5mL of water to concentration of 100mg/mL for method development. A 3g of the sample matrix was also spike with a calculated amount of the working standards solution to concentrations between 50 and 100 µg/kg used for method optimization and validation studies. A concentration ranges of 10 -500µg/kg was prepared directly in the matrix for the calibration purpose and method validation.

3.3.2 Sample Collection

Two types of beans sample were purchased from local sellers in 5 wholesale markets in Ilorin metropolis. The samples were not collected in plastic sample containers to prevent contamination with phthalate ester, they were collected in glass bottles with tight covers to protect them from moisture and contaminations. Exactly 1 kg of each of the two types of beans was purchased from five different sellers in each of the market and composited to represent one sample of the foodstuff. All samples were code named and labeled accordingly. The samples were taken to the laboratory and stored in a refrigerator at 4 °C until analysis. The samples used for method validation, calibration and recovery studies were first analyzed to ensure the absence of the target pesticide residues.

3.3.3 Sample Preparation

The samples were cleaned by picking out stones and other extraneous materials. For AOAC (2007) QuEChERS method, 200/g of each beans samples was homogenized and grinded. Then 15g aliquot of the homogenized sample was weighed and transferred into 50 ml polypropylene tube, then spiked with a calculated amount of pesticide standard mixture, and was left to stand for 1hour at room temperature. Then 15ml of 1% acetic acid in acetonitrile was added, followed by addition of 0.1 ml of triphenyl phosphate (Internal standard) and manually shaked for 1 minute. This was followed by addition of6g of anhydrousmagnesium sulphateand 1.5g of sodium acetate. The mixture was shaken vigorously for 1min and the mixture was centrifuged for 5 minute at 5000rpm. After centrifugation, the supernatant was collected and 5 ml of the supernatant was transferred into a centrifuge tube, followed by addition of 150 mg of $MgSO_4$ and 250 mg of primary secondary amine (PSA). The mixture was shaken vigorously for 30 sec, and centrifuged at 4000 rpm for 5 minutes. The final extract

was carefully transferred to amber colour glass vial. An aliquot of 20 μL of the final extract was then injected into GC-MS for separation and quantification.

3.4 GC-MS Analysis

The analysis of pesticides was carried out with CTC CombiPALautosampler coupled to a GC-MS (Shimadzu QP2010Series) and operated in the split/splitless mode at an injection temperature of 260 °C. The separation of target analytes were achieved on a DB-5MS fused capillary column containing 5 % diphenyl and 95 % dimethylpolysiloxane (30 m x 0.25 mm i.d. 0.25 µm film thickness). The injection port of the GC was equipped with a high-pressure Merlin Microsealseptumless injection kit and a silanized narrow bore liner (78.5 x 6.5 mm o.d x 0.75 mm i.d). Helium (carrier gas, 99.999 % purity) was set to a constant flow rate of 1.3 mL/min with linear velocity of 42 cm/sec. The MS operation condition includes transfer line of 300 °C, ion source of 200 °C, electron ionization (EI) of 70 eV. The optimization of methods was done in scan mode while quantitation was done in selected ion monitoring (SIM) mode. A target ion (most abundance ion) and two other reference ions were monitored for the target analytes.

3.5 Preparation of Calibration Curve

The analytical figures of merit were validated using internal standard prepared in a matrix match calibration standard. The calibration curve of each pesticide was constructed using matrix sample spiked at eight different concentrations with the working standard solution containing the internal standards. The mixture of standard solution of the pesticide was run in GCMS to obtain their retention time under the set chromatography condition. The concentration prepared ranged from 1 to 500/μg/kg and the peak area obtained for each analyte and the external standard was used to construct a calibration curve by plotting the ratio of peak area of each analyte as a function of concentration. Each concentration point was analyzed in triplicate in three different sample matrices. The precision, accuracy, selectivity and sensitivity,

limit of detection (LOD)|, limit of quantitation (LOQ), limit of quantitation (LOQ) and the average recovery were determined.

3.6Method Development and Validation of Analytical Methodology

The analytical figures of merit were validated using internal standard prepared in matrix match calibration standard. The calibration curve of each pesticide was constructed using matrix sample spiked at five different concentrations with the working standard solution. The concentration prepared ranged from 0.25 to 1000 μg/kg and the peak area ratio which is ratio of the peak area of analytes to the peak area internal standard was plotted against the concentration of analytes. Each concentration point was analyzed in triplicate in three different sample matrices. The precision, accuracy, selectivity and sensitivity, limit of detection (LOD), limit of quantitation (LOQ), limit of quantitation (LOQ) and the average recovery were determined.

3.7 Effect of Heat

To determine the effect of heat, 200g of each beans sample was boiled in water at 100°C until is it good for consumption, they were then grinded into paste using a porcelain mortar and pestle. An aliquot of 2g of each grinded sample was taken for QuEChERS procedure described above.

3.8 Effect of Washing

To determine the effect of washing on pesticide residues, 200g of each beans sample were washed withrunning tap water for 30 min. The beans sample were then air dried for 2 hrs and then grinded into powder using a porcelain mortar and pestle, an aliquot of 2g of each grinded sample was taken for QuEChERS procedure.

3.9 Data Analysis

The data collected was analyzed using both quantitative and quantitative techniques. The data was analyzed using Microsoft excel to draw graph and for analysis of variance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Coding of Sample

The samples were assigned codes based on the markets where they were purchased. As shown in Table 4.1, two different beans samples (brown and white) were purchased from 5 markets (Baboka, Mandate, Ipata, Ita-Amo, Oja Oba) in Ilorin metropolis.

Table 4.1: Coding of beans samples according to market. The sources of each beans samples were determined through oral interview with the traders.

Code	Market	Type	Source
BBM	Baboko	Brown	Sokoto
BMM	Mandate	Brown	Sokoto
BIM	Ipata	Brown	Kano
BIAM	Ita Ama	Brown	Niger
BOM	Oja Oba	Brown	Niger
WBM	Baboko	White	Kano
WMM	Mandate	White	Niger
WIM	Ipata	White	Niger
WIAM	Ita Ama	White	Niger
WOM	Oja Oba	White	Kano

N.B. BBM: Brown samplefromBaboko market WBM: White sample from Baboko market
BMM: Brown sample from Mandate market WMM: White sample from Mandate market
BIM: Brown sample from Ipata marketWIM: White sample from Ipata market
BIAM: Brown sample from Ita ama market WIAM: White sample from Ita Ama market
BOM: Brown sample from Oja Oba marketWOM: White sample from Oja Oba market

4.2 Calibration Curve

Calibration curve is a plot of how the instrumental response changes with the concentration of the analyte. A series of standards across a range of concentration near the expected concentration of analyte in the unknown is prepared. The mixed standard solution of the pesticides were analyzed to obtain their retention time and was used to calibrate the calibration curve (Fig 4.1 and Fig 4.2).

Fig 4.1 shows that the regression equation of bifethrin is y= 0.0273x + 0.031 with R^2 of 0.9997 while the regression equation in dichlorovos is y = 0.0128x + 0.0458 with R^2 of 0.9996 as shown in Fig 4.2.

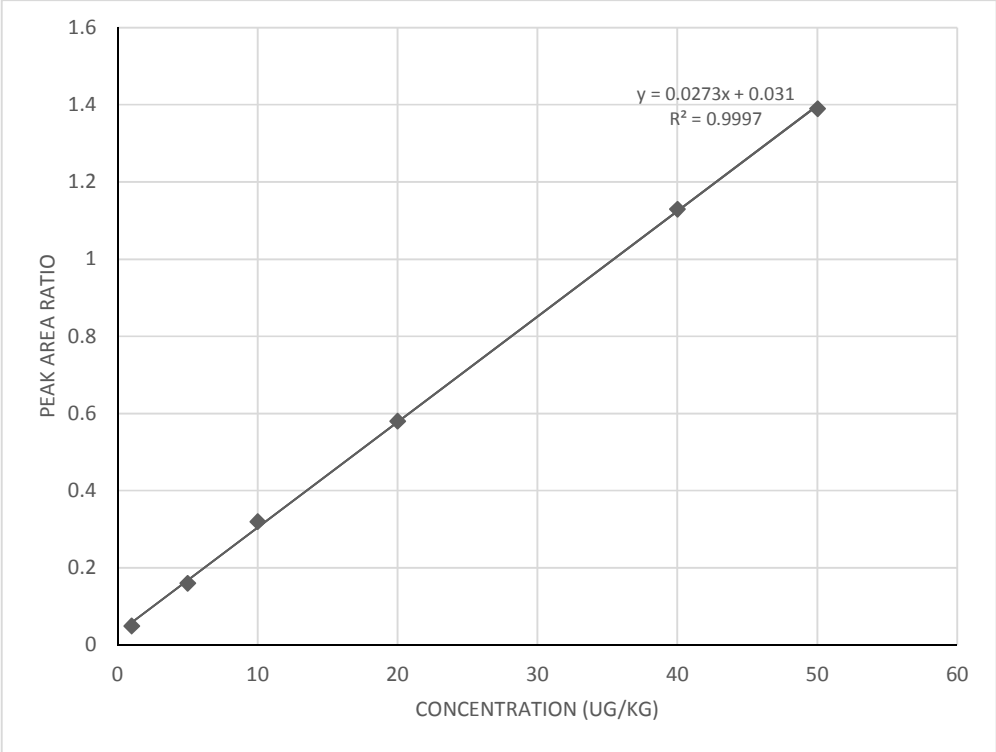


fig. 4.1: Calibration Curve for Bifenthrin

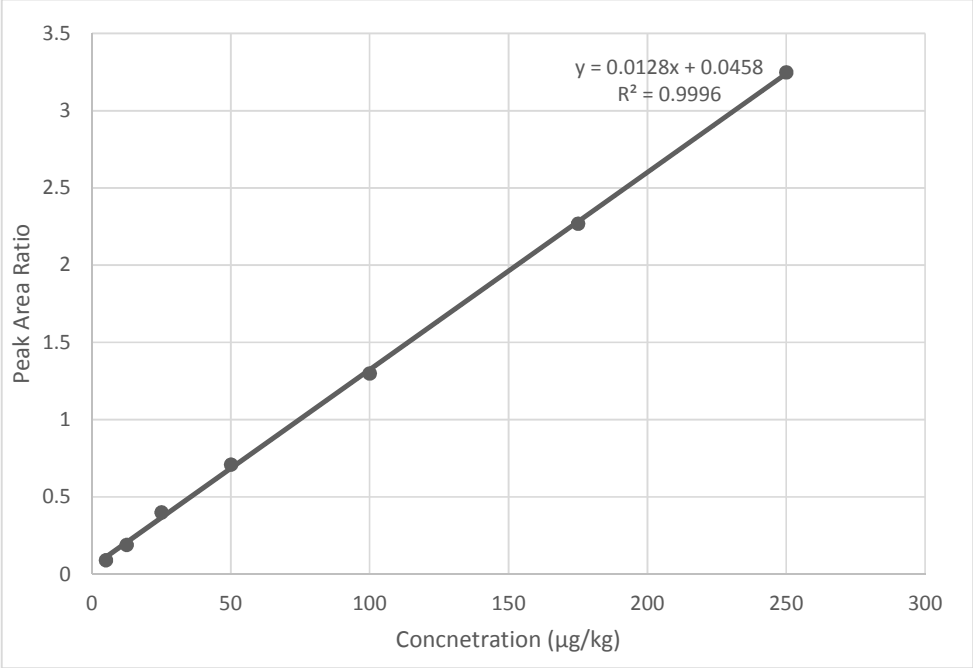


Fig. 4.2: Calibration Curve for Dichlorvos

4.3 Retention Time and Correlation Coefficient

The retention time, correlation coefficient and the target and selected ion monitoring are presented in Table 4.2 below.The Retention time is the amount of time a compound spends on the column after it has been injected. It is the time clasped between sample introduction (beginning of chromatogram) and the maximum signal of the given compound at the detector.

Table 4.2: Retention time (RT), correlation coefficient and selected monitored ions of thePesticides

Pesticide	**RT**	**r^2**	**Monitored ion (m/z)**
Dichlorovos	8.37	0.9996	109, 185, 220
Bifenthrin	60.02	0.9997	165, 181, 342

N.B.: RT: Retention time, r^2: Correlation Coefficient

4.4 Method Validation

In this study, the figures ofmerit of analytical methodology of the developed method was validated in terms oflinearity, accuracy, intra-day and inter-day precision, limit of detection (LOD) and limit ofquantification (LOQ) using the AOAC modified QuEChERS.

4.4.1 Linearity Curve

The linearity of an analytical method is the ability to produce a measured value (chromatographic peak area) that a directly proportional to the concentration of the analyte in the sample matrices within a given range. The determination of linearity is used in connection with the formulation of the calibration curve. The range within which the measured response is directly proportional to the concentration of the analytes is called the linear range, which is the interval between the lower and upper calibration points of the spiked sample. The peak area ratio which is ratio of the peak area of analytes to the peak area internal standard was plotted against the concentration of analytes. Table 4.3 shows the calibration parameters. The calibration curves were linear over the tested concentration range. The correlation coefficients (R^2) were greater than 0.99 for all the investigated pesticides.

Table 4.3: Linearity range, calibration equation and correlation coefficient in beanssamples

Pesticide	**Linearity range (µg/kg)**	**r^2**	**Calibration equation**
Dichlorvos	1-1000	0.9996	y = 0.0128x + 0.0458
Bifenthrin	0.25 -1000	0.9997	y = 0.0273x + 0.031

r^2: Correlation coefficient

4.4.2 Accuracy and Precision

Accuracy is determined based on the analysis of sample spiked with a known amount of pesticide and comparing the measured value with the spiked value. The accuracy is reported as the percent recovery by the analysis of a known added amount of pesticides in the sample matrix while, Precision is the closeness of agreement between a series of independent measurement obtained when an analytical method is applied in replicate to multiple sampling of homologous samples. It is usually specified in terms of the relative standard deviation (RSD).

The accuracy, inter-day and the intra-day precision were determined by spiking the samples at three concentration levels and three replicate analyses were run for each concentration on the same day. The intra-day precision (n = 3) was estimated by performing three extractions in a single day, and inter-day precision (n = 9) was estimated based on three extractions per day for three days, while the accuracy was reported in terms of the average recoveries of the spiked sample at different concentration levels.

A one-way single factor ANOVA, was used to estimate the variance, which gives the total sum of square, between group mean square (BMS) and within group mean square (WMS). The BMS estimate the variance associated with the intra-day precision (within-day) and inner-day precision (between-day) variances. The inter- and intra-day RSD (n = 3) obtained were 5.81 % and 6.59 % respectively.

$$\%\ RSD\ (Intra-day) = \frac{\sqrt{WMS}}{Average\ Relative\ Recovery} \text{x } 100$$

$$\%\ RSD\ (Inter-day) = \frac{\sqrt{\left(\frac{BMS-WMS}{N}\right) + WMS}}{Average\ Relative\ Recovery} \text{x } 100$$

4.4.3 Recovery

The efficiency and accuracy of any extraction technique is determined based on the average recovery. The recovery is determined as relative recovery, which involves the analysis of known amounts of analytes spiked into the sample matrix, and comparing thechromatographic peak area obtained with the chromatographic peak area obtained whenanalyzing a standard solution of the same concentration under the same experimentalconditions.

Table 4.5 below shows the precision (inter- and intra-day RSD), accuracy and the AOAC method in spiked beans sample.As shown in Table 4.4 below, the relative recovery of the spiked bean sample ranged from 91.56 to 105.33 % for dichlorvos and 101.99 – 104.94 % in bifenthrin, with RSD ranging from 8.11- 13.92 and 7.39 – 9.98% respectively. The results obtained for the precision and accuracy study are therefore in accordance with the acceptable practice and the results are satisfactory for determination of the targetpesticides in the complex sample matrices with no significant matrix interference. All the parameters validated in this study were based on the methodvalidation requirements of the European Union (SANCO, 2011).

Table 4.4: Accuracy (% Recovery) and Precision (% RSD) of AOAC method

Pesticide	**Added (µg/kg)**	**Accuracy (%)**	**RSD (%)**
Dichlorvos	50	91.56	13.92
	100	105.33	8.11
Bifenthrin	50	104.94	7.39
	100	101.99	9.89

4.4.4 Selectivity

Selectivity is defined according to the ICH document as the ability to assess the presence of an analyte unequivocally, in the presence of other interfering components, such as impurities, matrix components and/or degradation products which are expected to be present.

It also describes the ability of an analytical instrument to produce a signal which represents the target analyte and not the interfering component.

In this study, the selectivity was determined by extracting a blank matrix containing the internal standard and a sample spiked with the target analyte. The resulting chromatograms are as shown in Figure 4.3, which indicates a good selectivity with no interference at the retention times of the target analytes. Triphenylphospate acts as an internal standard.

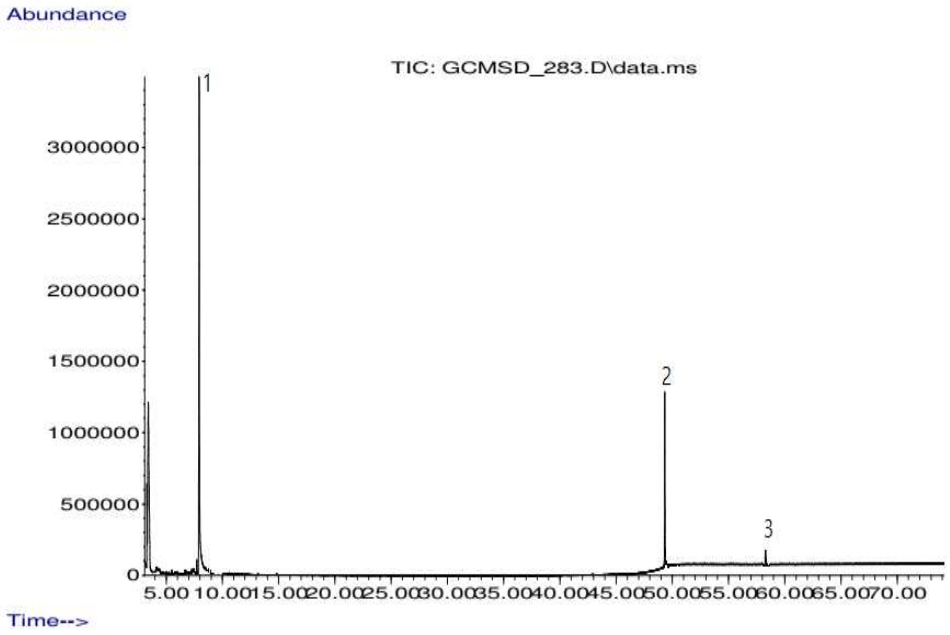


Figure 4.3: Chromatograms of beans sample spiked with mixed standard of dichlrovos and bifenthrin1: Dichlorvos2: Triphenylphosphate3: Bifenthrin

4.4.5Limit of Quantitation and Limit of Detection

The limit of quantitation (LOQ) is defined as the lowest concentration of analytes that can be quantitatively determine with an acceptable level of accuracy and precision, while the limit of detection(LOD) is defined as the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value under the optimized experimental conditions.

The LOQ is estimated based on the signal-to-noise ratio of 10:1, while the LOD is estimated based on the signal-to-noise ratio of 3:1. The LOQ and LOD were estimated using the following equation:

$$LOQ = \frac{10\sigma}{S}$$

$$LOD = \frac{3\sigma}{S}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve. In this study, the LOQ and LOD were determined using the standard deviation of the y-intercept of the regression line. The LOQ and LOD obtained are as shown in Table 4.5 below. The LOQ was found to be 37.91 and 8.87 µg/kgfor dichlrovos and bifenthron respectively, while LOD was found to be 11.37 and 2.66µg/kgrespectively.

Table 4.5: Limit of quantitation and limit of detection of AOAC method

Pesticide	**LOQ (µg/kg)**	**LOD(µg/kg)**
Dichlorvos	37.91	11.37
Bifenthrin	8.87	2.66

N.B. LOQ: Limit of Quantitation; LOD: Limit of Detection

4.5 Pesticide Residue Content of Samples

The results of analysis of beans samples obtained from 5 different markets in Ilorin metropolis using AOAC QuEChERS method are presented in Table 4.6 below. The level of pesticide residues present in food depends on the amount of active ingredient used and also on the susceptibility of the food item to infestation. The results affirmed that, all the beans samples analyzed contain dichlorvos, while few samples contain bifenthrin, and there are higher concentration of pesticides in the white beans compared to the brown beans. The residue content of dichlorvos was found to be higher than the maximum residue levels (MRL), while bifenthrin was found to be lower than the MRL. The presence of dichlorvos which is widely

used by Nigerian beans farmers on all the beans samples might be due to the susceptibility of beans to infestation by weevils (pre- and post-harvest). Bifenthrin on the other is used only as post-harvest treatment. Therefore, in order to maintain good quality, and avoid economic losses, beans is subjected to pre- and post-harvest treatment with pesticides (Ogah *et al*., 2012). Higher dichlorvos level has led to several rejections of beans exported to European countries due to the presence of pesticide residues at concentration higher than the MRL(Izuaka, 2020).

Table 4.6: Pesticide residue content of beans samples (n = 3)

Code	Dichlorvos (µg/kg)	Bifenthrin (µg/kg)
BBM	171.25±11.45	7.52±1.42
BMM	137.64±7.18	5.24±3.12
BIM	152.22±10.31	7.62±2.28
BIAM	175.64±4.53	n.d
BOM	190.27±12.12	n.d
WBM	167.07±10.32	6.26±3.12
WMM	163.10±13.81	n.d
WIM	137.00±4.19	n.d
WIAM	139.80±4.73	n.d
WOM	145.74±7.12	3.84±1.80
MRL	100	10

N.B. n.d: not detected or below limit of detection
BBM: Brown sample from Baboko market
BMM: Brown sample from Mandate market
BIM: Brown sample from Ipata market
BIAM: Brown sample from Ita ama market
BOM: Brown sample from Oja Oba market
MRL: Maximum Residue Level.
WBM: White sample from Baboko market
WMM: White sample from Mandate market
WIM: White sample from Ipata market
WIAM: White sample from Ita Ama market
WOM: White sample from Oja Oba market

4.6 Effect of Heat on Pesticide Residues

The effect of boiling on the concentration of dichlorvos and bifenthrin residues in beans was determined by cooking the beans samples in water until it is good for consumption. The result is as presented in Table 4.7.

It can be observed that boiling of beans sample reduced the pesticide residues content of the samples by 34.42 – 51.76 % in dichlorvos, while it reduced the bifenthrin residue by11.45 – 28.65 %. The higher reduction in dichlorvos content was as a result of its higher octanol-water coefficients and high solubility water used to boil the beans samples.

The processing of agricultural products before consumption has been observed to have considerable effect on the residue content. It was found that the pesticide residue content reduced with boiling. This agree with previous studies of Rasmussen (2003), who reported reduction in the residue content due to boiling. The result showed that boiling has significant effect on pesticide residues in food.

Table 4.7: Residue content of beans samples before and after boiling

Code	Dichlorvos (µg/kg)			Bifenthrin (µg/kg)		
	Uncooked	**Cooked**	**% Reduction**	**Uncooked**	**Cooked**	**% Reduction**
BBM	171.25	110.39	35.54	7.52	5.43	27.79
BMM	137.64	79.50	42.24	5.24	4.64	11.45
BIM	152.22	95.09	37.53	7.62	5.92	22.31
BIAM	175.64	90.15	48.67	n.d	n.d	0
BOM	190.27	96.73	49.16	n.d	n.d	0
WBM	147.07	72.62	50.62	6.26	4.67	25.40
WMM	136.10	74.16	45.51	n.d	n.d	0
WIM	137.00	73.07	46.66	n.d	n.d	0
WIAM	139.80	67.44	51.76	n.d	n.d	0
WOM	145.74	95.58	34.42	3.84	2.74	28.65

N.B. n.d: not detected or below limit of detection MRL: Maximum Residue Level.
BBM: Brown sample from Baboko market WBM: White sample from Baboko market
BMM: Brown sample from Mandate market WMM: White sample from Mandate market
BIM: Brown sample from Ipata market WIM: White sample from Ipata market
BIAM: Brown sample from Ita ama market WIAM: White sample from Ita Ama market
BOM: Brown sample from Oja Oba market WOM: White sample from Oja Oba market

4.7 Effect of Washing

The effect of washing on residue content of the beans sample was determined by washing before extraction and analysis. The result (Table 4.8), showed a reduction of between 52.43 and 70.30% in dichlorvos and 8.02 and 20.03 in bifenthrin. Washing was found to be

more effective that boiling for removing pesticide residues in beans. This could be as result of the fact that washing is more dynamic than boiling, as the water is continually being replaced during washing. The lower reduction is pesticide residues after boiling compared to washing could be as a result of the volatility of the pesticide residues.

Table 4.8: Residue content of beans samples before and after washing

Code	Dichlorvos (µg/kg)			Bifenthrin (µg/kg)		
	Unwashed	**Washed**	**% Reduction**	**Unwashed**	**Washed**	**% Reduction**
BBM	171.25	71.43	58.29	7.52	6.59	12.37
BMM	137.64	64.23	53.33	5.24	4.82	8.02
BIM	152.22	52.67	65.40	7.62	6.14	19.42
BIAM	175.64	55.93	68.16	n.d	n.d	0
BOM	190.27	56.51	70.30	n.d	n.d	0
WBM	147.07	44.62	69.66	6.26	5.01	19.96
WMM	136.10	64.61	52.53	n.d	n.d	0
WIM	137.00	57.73	57.86	n.d	n.d	0
WIAM	139.80	52.43	52.43	n.d	n.d	0
WOM	145.74	53.82	53.82	3.84	3.04	20.83

N.B. n.d: not detected or below limit of detection MRL: Maximum Residue Level.
BBM: Brown sample from Baboko market WBM: White sample from Baboko market
BMM: Brown sample from Mandate market WMM: White sample from Mandate market
BIM: Brown sample from Ipata market WIM: White sample from Ipata market
BIAM: Brown sample from Ita ama market WIAM: White sample from Ita Ama market
BOM: Brown sample from Oja Oba market WOM: White sample from Oja Oba market

CHAPTER FIVE

5.0Conclusion

The study have shown that beans farmers in Nigeria used the investigated pesticides in a concentration higher than the recommended dosage of active ingredient per hectare of land. This resulted in concentration greater than the MRL (which is the maximum concentration of pesticide residue that is legally permitted to remain in food after it has been treated with the pesticide.

The need for frequent monitoring of pesticide residue in food commodities has resulted in the development of different extraction techniques. The application of QuEChERS has shown to be accurate, precise, time saving and efficient for the analysis of pesticide residues in food sample. The results if this study show that beans samples sold in Ilorin metropolis contain pesticide residue which was found to be higher than the maximum concentration legally permitted to be on the food. It can be concluded from this studies that most of the beans sold in Ilorin metropolis were bought from Niger State, while few are from Kano and Sokoto States.

5.1Recommendations

There is need for the relevant agencies of government such ad National Agency forFood Drug Administration and Control (NAFDAC), Standard Organization Nigeria (SON) and the Federal Ministry of Agriculture to strictly control the importation, sales and use of the pesticide. On the farm monitoring is also recommended in order to ensure that farmers engage in good agricultural practices (GAP) on the use of pesticide, and ensures that the food we eat does not pose any health risk. Further work should be carried out on a wide range of pesticides and foodstuff, and the daily intake of pesticide residue and consumption pattern is determined.

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