

**COMPARISON OF PESTICIDE RESIDUE IN BEANS (*Phaseolus vulgaris*)**

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**SCHOOL OF BASIC MEDICAL SCIENCES**

**UNIVERSITY OF BENIN,**

**BENIN CITY.**

**MARCH, 2025**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL  
BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF  
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**CERTIFICATION**

We the undersigned hereby certify that **OKOSODO VALENTINE OSEMUDIAMEN** carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the reward of Bachelors/Masters of Science Degree (B.Sc) in Medical Biochemistry.

Signed

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**EXTERNAL EXAMINER**

**Date**

## **DEDICATION**

I dedicate this project to God Almighty, my source of strength, inspiration, wisdom, knowledge and understanding and to my lecturers who have taught me up to this point in my academic pursuit, equipping me with knowledge for both self and societal development.

## ACKNOWLEDGEMENT

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## ABSTRACT

The contamination of stored food products by pesticide residues has become a major concern due to its potential health risks and environmental implications. Pesticides, widely used for pest control in agriculture and storage facilities, can leave residues that persist in food products, leading to human exposure through consumption. This study investigates the presence and concentration of pesticide residues in stored beans using High-Performance Liquid Chromatography (HPLC) analysis, focusing on two commonly used pesticides—dichlorvos and cypermethrin. These pesticides are frequently applied to stored grains to protect against insect infestation, but their residues may pose significant risks if present in excessive amounts.

In this research, samples of stored beans were collected from multiple storage facilities to assess pesticide contamination levels. The HPLC method was optimized for high sensitivity and precision, ensuring accurate detection and quantification of dichlorvos and cypermethrin residues. The analysis revealed varying concentrations of pesticide residues across different samples, with some exceeding the maximum residue limits (MRLs) established by regulatory authorities. These findings highlight the potential risks associated with consuming contaminated beans, particularly concerning chronic exposure to pesticide residues, which has been linked to adverse health effects such as neurological disorders, endocrine disruption, and carcinogenicity.

The study also explores the regulatory framework governing pesticide residue levels in food products, emphasizing the necessity of strict compliance with food safety guidelines. The findings underscore the importance of regular monitoring and enforcement of pesticide regulations to prevent excessive contamination of food commodities. Additionally, the study discusses the impact of storage conditions on pesticide persistence, noting that factors such as temperature, humidity, and storage duration can influence residue degradation or accumulation.

Given the health risks associated with pesticide residues, this research advocates for the adoption of safer and more sustainable pest control strategies in food storage. Integrated pest management (IPM) approaches, including biological control methods, improved storage techniques, and reduced chemical dependency, should be prioritized to minimize the presence of harmful

residues in food products. The study also suggests the need for further research into alternative storage practices that effectively preserve food quality while ensuring consumer safety.

In conclusion, the detection of dichlorvos and cypermethrin residues in stored beans raises significant food safety concerns. The results of this study emphasize the urgent need for improved monitoring, stricter regulation, and the promotion of alternative pest control methods to ensure the safety and sustainability of food storage systems. By implementing proactive measures, policymakers, regulatory agencies, and stakeholders in the agricultural sector can contribute to minimizing pesticide contamination and safeguarding public health.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of Study

Beans (*Phaseolus vulgaris*) are a vital legume crop widely cultivated in tropical and subtropical regions, offering essential nutrients and playing a key role in soil enrichment through nitrogen fixation (Martin *et al.*, 2017).

Despite their importance, bean production faces significant threats from pests, prompting the frequent use of synthetic pesticides. These chemicals, while effective, can result in harmful residues in the final food product (Adjei *et al.*, 2020).

In many developing countries, pesticide residue levels often surpass internationally accepted limits, raising public health concerns (Thompson *et al.*, 2016).

This study investigates pesticide residues in beans from various sources to inform safer agricultural and food handling practices (Kusi *et al.*, 2021).

### 1.2 Justification of the Study

Beans are a critical protein source for many low- income populations and a staple in numerous diets globally (Jones *et al.*, 2019). Ensuring the safety of beans from pesticide contamination is essential for public health, particularly due to the potential chronic effects of long- term exposure (Thompson *et al.*, 2016).

Even with established regulations, residue levels in beans often exceed permissible thresholds, posing a threat to consumers (Adjei et al., 2020). A clear understanding of residue levels across different supply chains is necessary to evaluate contamination risks and uphold food safety standards (FAO/WHO, 2020).

Comparative studies serve as a foundation for enforcing safe pesticide use and protection public health (Boateng et al., 2021). Additionally, identifying contamination hotspots allows for targeted regulatory and educational interventions (Kusi *et al.*, 2021)

### **1.3 Aim of the Study**

This study aims to compare pesticide residue levels in *Phaseolus vulgaris* (beans) from various sources using validated analytical techniques. The specific objectives are to:

1. Quantify pesticide residues using GC-MS and HPLC (Zhang *et al.*, 2020).
2. Compare residue levels in beans from different markets, farms, and storage facilities (Owusu *et al.*, 2021).
3. Evaluate compliance with maximum residue limits (MRLs) set by FAO/WHO, EPA, and EFSA (FAO/WHO, 2020).
4. Assess potential health risks linked to detected pesticide residues (Thompson, *et al.*, 2016).
5. Recommend safer pest management approaches such as integrated pest management (IPM) and organic alternatives (Kusi *et al.*, 2021).

Through this study, insights into the extent of pesticide contamination in beans will support efforts to improve food safety, ensure regulatory compliance, and safeguard consumer health (Boateng *et al.*, 2021).

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Overview of Beans (*Phaseolus vulgaris*)

Beans (*Phaseolus vulgaris*) is a vital leguminous crop cultivated extensively in tropical and subtropical regions, particularly in Africa, Asia, and South America. It serves as a major source of dietary protein, essential vitamins, and minerals, making it an important staple food for many populations (Ehlers & Hall, 1997; Timko et al., 2007).

Additionally, beans contributes to soil fertility through biological nitrogen fixation, thereby enhancing agricultural sustainability (Ronner & Giller, 2013). Its remarkable tolerance to drought conditions makes it a valuable crop for smallholder farmers, supporting both food security and livestock feed systems (Fatokun et al., 2018; Kouakou et al., 2020).

Moreover, bean residues can be used as green manure, further enriching soil health and promoting sustainable farming practices (Singh & Sharma, 2021).

##### 2.1.1 General Information on Beans

Common bean (*Phaseolus vulgaris*) is a significant leguminous crop cultivated in tropical and subtropical regions, serving as a major source of protein, essential vitamins, and minerals (Broughton et al., 2003; Pérez et al., 2020). It plays a crucial role in improving soil fertility through nitrogen fixation, making it beneficial for sustainable agriculture (Beebe et al., 2013). The crop exhibits moderate drought tolerance, allowing it to adapt to diverse climatic conditions and support food security, particularly in smallholder farming systems (Blair et al., 2010; Rao et

al., 2017). Additionally, common bean is widely utilized as food, livestock feed, and green manure, further enhancing its agricultural and economic value (Van Schoonhoven & Voysest, 2010; Akibode & Maredia, 2011).

### **2.1.2 Description of Beans**

Common bean (*Phaseolus vulgaris*) is a highly diverse legume with variations in plant architecture, growth habits, and seed characteristics, including differences in size, shape, and color (Singh et al., 1991; Gepts et al., 2008). It thrives in warm environments but is sensitive to extreme drought and poor soil conditions, requiring well-drained soils for optimal growth (Beebe et al., 2013; Porch et al., 2013). The crop is primarily self-pollinating, with occasional cross-pollination facilitated by insects (Gioia et al., 2019). Its relatively short growth cycle allows for cultivation in regions with limited rainfall, making it an essential food crop for diverse agricultural systems (White et al., 1992; Rao et al., 2013).

## **2.2 Botanical Description of Beans**

Common bean (*Phaseolus vulgaris*) is a dicotyledonous plant characterized by distinct morphological adaptations (Gepts et al., 2008). It possesses a well-developed taproot system, which enhances its ability to access deep soil moisture and tolerate periods of drought (Beebe et al., 2013). The stems exhibit varying growth habits, ranging from determinate bush types to indeterminate climbing forms, which contribute to its adaptability in different farming systems (Singh, 1999; Porch et al., 2013). Its trifoliolate leaves facilitate efficient photosynthesis and environmental resilience (Blair et al., 2010), while its papilionaceous flowers support self-

pollination with occasional insect-mediated cross-pollination (Gioia et al., 2019). The plant produces elongated pods containing seeds that serve as a rich source of protein and carbohydrates, making it a crucial dietary staple (Broughton et al., 2003; Rao et al., 2013).

### **2.3. Taxonomical Classification of Beans**

The scientific classification of common bean is as follows:

- Kingdom: Plantae
- Phylum: Angiosperms
- Class: Eudicots
- Order: Fabales
- Family: Fabaceae
- Subfamily: Faboideae
- Genus: Phaseolus
- Species: Phaseolus vulgaris

Common bean includes multiple landraces and cultivars, with significant genetic diversity contributing to its adaptation to different environments.

### **2.4. Uses of Beans**

#### **1. Culinary:**

Common bean seeds, pods, and leaves are valuable sources of protein, fiber, and essential nutrients (Broughton et al., 2003; Messina, 2014).

#### **2. Agricultural:**

Beans contribute to soil fertility through nitrogen fixation and are widely used in intercropping systems to enhance crop diversity and productivity (Peoples et al., 2009; Beebe et al., 2013).

#### **2.4.1. Medical Benefits of Beans**

**Protein:** A rich source of plant-based protein, making it a crucial dietary component (Mitchell et al., 2009).

**Diabetes:** Helps regulate blood sugar levels due to its low glycemic index and high fiber content (Marinangeli & Jones, 2011).

**Heart Health:** Contains flavonoids and soluble fiber that help reduce cholesterol levels and lower the risk of cardiovascular diseases (Darmadi-Blackberry et al., 2004).

**Weight Management:** High fiber promotes satiety, aiding in appetite control and weight regulation (McCrorry et al., 2010).

**Antioxidants:** Rich in polyphenols and flavonoids that combat oxidative stress and inflammation (Ranilla et al., 2007).

**Digestive Health:** Supports gut health by promoting beneficial gut bacteria and improving digestion (Slavin, 2013).

#### **2.4.2. Non-Medical Benefits of Beans**

**Food Security:** Adaptable to different climatic conditions and essential for protein intake in developing regions (Beebe et al., 2013).

**Economic Benefits:** Supports smallholder farmers and is a high-value crop in both local and international markets (Giller, 2001).

**Cultural Significance:** A staple in many traditional cuisines worldwide, including Latin American, African, and Asian dishes (Gepts & Debouck, 1991).

**Environmental Sustainability:** Improves soil fertility through nitrogen fixation, reducing the need for synthetic fertilizers (Peoples et al., 2009).

## **2.5 Storage of Beans**

### **2.5.1 Traditional Methods**

**Sun Drying:** Widely used in rural areas to reduce moisture content and prevent fungal growth, though it is highly weather-dependent (Cichy et al., 2015).

**Local Containers:** Beans are commonly stored in woven bags, clay pots, or wooden bins in dry, ventilated areas, but these methods offer limited pest protection (Baoua et al., 2014).

**Smoke-Drying:** Practiced in some regions to extend shelf life, though it can alter the beans' flavor, color, and texture (Murdock et al., 2012).

### **2.5.2 Modern Methods**

**Cold Storage:** Storing beans at temperatures between 5°C and 10°C helps maintain quality and prevents microbial spoilage (Vasconcelos et al., 2010).

**Hermetic Storage:** Airtight containers or plastic bags reduce oxygen levels, preventing insect infestation and moisture absorption (Baributsa et al., 2010).

**Chemical Preservatives:** Protectants like inert dusts or food-grade diatomaceous earth are used to control insect pests without compromising seed quality (Golob, 2002).

**Modified Atmosphere Storage (MAS):** Regulating oxygen and carbon dioxide levels in storage environments helps extend shelf life and prevent pest infestation (Navarro et al., 2012).

## 2.6 Pesticides

A pesticide is a chemical or biological substance used to control pests, including insects, weeds, fungi, and rodents. It is essential in agriculture to protect crops such as common bean (*Phaseolus vulgaris*) from pests and diseases, ultimately improving yield and quality (Cooper & Dobson, 2007; Matthews, 2018). Pesticides help manage common bean pests such as bean weevils (*Acanthoscelides obtectus*) and aphids (*Aphis fabae*), reducing post-harvest losses and enhancing storage longevity (Schmale et al., 2002; Abate et al., 2000). However, concerns regarding pesticide resistance, environmental pollution, and health risks have led to increased promotion of integrated pest management (IPM) strategies for more sustainable pest control (Popp et al., 2013).

### 2.6.1 Classification of Pesticides

Pesticides are substances used to control a wide range of pests, including insects, fungi, weeds, and rodents. They are classified based on their target organisms, chemical composition, mode of action, and environmental persistence (Ware & Whitacre, 2004; Mansour et al., 2018). The main categories include:

- **Insecticides:** Used to control insect pests, they include organophosphates, which inhibit acetylcholinesterase activity in insects (Casida & Durkin, 2013), and pyrethroids, which disrupt sodium channels in insect nervous systems (Soderlund, 2012).

- **Herbicides:** Chemicals that control weeds, categorized into selective herbicides, which target specific plant species, and non-selective herbicides, which kill a broad spectrum of plants, such as glyphosate (Duke & Powles, 2008).
- **Fungicides:** Used to manage fungal diseases in crops, they include systemic fungicides like azoxystrobin and contact fungicides such as copper-based compounds (Hewitt, 1998; Brent & Hollomon, 2007).
- **Rodenticides:** Designed to control rodent populations, they include anticoagulants like bromadiolone, which interfere with blood clotting (Buckle & Eason, 2015).

Pesticides can also be classified by their persistence in the environment:

- **Persistent pesticides:** Such as Dichlorodiphenyltrichloroethane (DDT), remain active for long periods, potentially causing long-term environmental contamination (van den Berg, 2009).
- **Non-persistent pesticides:** Such as pyrethroids, degrade quickly, reducing long-term ecological risks (Goulson, 2013).

Additionally, newer environmentally friendly alternatives, such as biopesticides derived from natural sources like *Bacillus thuringiensis* and neem extracts, offer effective pest control with minimal environmental impact (Dubey et al., 2010; Copping & Menn, 2000). The careful selection and application of pesticides are essential to balancing pest management with environmental and human health considerations.

## 2.6.2 Mode of Action of Pesticides in Beans

Pesticides used in common bean (*Phaseolus vulgaris*) farming, including insecticides, herbicides, fungicides, and nematicides, function through various mechanisms to control pests and diseases that threaten yield and quality.

1. **Insecticides:** Target common bean pests such as bean weevils (*Acanthoscelides obtectus*) and aphids (*Aphis fabae*). Neurotoxic insecticides (e.g., organophosphates and pyrethroids) interfere with nerve signal transmission, leading to paralysis and death (Casida & Durkin, 2013). Systemic insecticides, absorbed by plant tissues, poison insects upon ingestion (Nauen et al., 2001). Insect growth regulators disrupt molting and reproduction, preventing pest population growth (Smagghe et al., 2012).

2. **Herbicides:** Control weeds competing with beans for nutrients and water. Selective herbicides target specific weed species by disrupting essential processes, such as glyphosate inhibiting amino acid synthesis (Duke & Powles, 2008). Non-selective herbicides, like paraquat, damage all plant cells by disrupting photosynthesis (Grossmann, 2010).

3. **Fungicides:** Manage fungal diseases such as anthracnose and rust. Systemic fungicides, like azoxystrobin, penetrate plant tissues to inhibit fungal reproduction (Oliver & Hewitt, 2014), while contact fungicides, such as copper-based compounds, eliminate fungal spores on the plant surface (Reis et al., 2019).

4. **Nematicides:** Protect beans from root-knot nematodes (*Meloidogyne spp.*), which damage root systems. Contact nematicides kill nematodes upon exposure (Chitwood, 2003), while soil fumigants like methyl bromide eradicate soil-borne nematodes (Zasada et al., 2010).

## 2.7 Pesticide Control in Cultivation of Beans (*Phaseolus vulgaris*)

Common bean cultivation faces threats from pests, fungi, and weeds, necessitating effective pesticide management strategies (Schmale et al., 2002).

1. **Insect Pest Control:** Bean weevils and aphids are managed using synthetic insecticides like cypermethrin, though overuse can lead to resistance (Abate & Ampofo, 1996). Biopesticides, including neem extract and *Bacillus thuringiensis* (Bt), offer sustainable alternatives (Mohan et al., 2007).

2. **Fungal Disease Management:** Fungal infections significantly reduce yields. Chemical fungicides such as carbendazim and mancozeb are commonly used (Twizeyimana et al., 2007), while biocontrol agents like *Trichoderma harzianum* enhance disease resistance (Howell, 2003).

3. **Weed Management:** Herbicides like pendimethalin and metolachlor help suppress weed growth, while cultural methods such as intercropping minimize reliance on herbicides (Fernández-Aparicio et al., 2016).

4. **Integrated Pest Management (IPM):** Combines chemical, biological, and cultural practices to ensure effective pest control while reducing environmental risks (Popp et al., 2013).

### 2.7.1 Health Implications of Pesticide Residues in Beans

Pesticide residues in beans pose significant health risks, potentially leading to acute and chronic conditions.

1. **Acute Toxicity:** High pesticide exposure, especially from organophosphates, can cause nausea, dizziness, and poisoning (Damalas & Eleftherohorinos, 2011).

2. **Chronic Health Effects:** Long-term pesticide exposure is associated with neurodevelopmental disorders and endocrine disruption (Mostafalou & Abdollahi, 2017).
3. **Carcinogenic Effects:** Pesticides like glyphosate and malathion have been linked to cancer risks (IARC, 2019; Mesnage & Antoniou, 2018).
4. **Immune System Impact:** Certain pesticides can suppress immune function, increasing susceptibility to infections (Corsini et al., 2008).
5. **Developmental Risks:** Prenatal pesticide exposure is associated with birth defects and male infertility (Kim et al., 2020).
6. **Environmental Impact:** Pesticide runoff contaminates soil and water sources, indirectly affecting human health (Aktar et al., 2009).
7. **Vulnerable Populations:** Children, pregnant women, and the elderly are more susceptible to pesticide-related health risks (World Health Organization, 2021).

## **2.8 Regulatory Standards and Safety Limits for Pesticide Residues in Beans**

Regulating pesticide residues in beans (*Phaseolus vulgaris*) is crucial to ensuring food safety and protecting public health. Regulatory agencies establish Maximum Residue Limits (MRLs) to prevent harmful pesticide exposure.

1. **Regulatory Standards:** MRLs are set to prevent excessive pesticide accumulation in beans, reducing health risks such as cancer and neurological disorders (FAO, 2021; Goulart et al., 2021).
2. **Global Frameworks:** Organizations such as the Codex Alimentarius, the European Food Safety Authority (EFSA), and the United States Environmental Protection Agency (EPA)

establish MRLs for pesticide residues in beans (FAO/WHO, 2021; EFSA, 2020; EPA, 2020). For instance, the MRL for chlorpyrifos in beans is set at 0.05 mg/kg by Codex Alimentarius (Codex Alimentarius, 2021).

3. **National Standards:** Individual countries, including Nigeria, India, and Brazil, have established their own MRLs for pesticides in beans. However, research has indicated that residue levels sometimes exceed safe limits due to misuse and poor regulation (Salami et al., 2021; Kaur et al., 2021).

4. **Monitoring and Enforcement:** Continuous pesticide residue monitoring by food safety agencies ensures compliance with MRLs and helps maintain food safety (EFSA, 2020; USDA, 2020).

5. **Health Risk Assessments:** MRLs are determined based on Acceptable Daily Intake (ADI) levels to prevent long-term health hazards (FAO/WHO, 2021).

6. **Challenges and Recommendations:** Enforcing MRLs in developing countries is challenging due to limited regulatory oversight, lack of farmer awareness, and improper pesticide application. Strengthening farmer training, enforcing stricter monitoring, and increasing consumer awareness programs are essential to improving compliance and safety (Akinmoladun et al., 2019; Ayodele et al., 2019).

## **CHAPTER THREE**

### **EQUIPMENT AND METHODOLOGY**

#### **3.1 MATERIALS**

##### **3.1.1 Apparatus**

The apparatus used during the research study were procured from a registered vendor and were at experimental standards at the point of purchase. They include:

1. Glassware and Labware
2. Glass rods
3. Conical bottom tubes (10 mL and 50 mL)
4. Microcentrifuge tubes
5. Pipettes (micropipettes, disposable pipette tips)
6. Magnetic stirrer
7. Syringes (with 0.45  $\mu\text{m}$  syringe membrane filters)
8. Vials (2 mL HPLC sample vials)
9. Shaker

##### **3.1.2 Equipment**

Major equipment used includes:

1. Shaking device – For sample homogenization
2. Magnetic stirrer – For controlled low-speed mixing
3. Centrifuge – Capable of 4000 rpm for phase separation
4. Evaporator (Rotary Evaporator or Nitrogen Evaporation System) – For solvent removal

5. HPLC System – Equipped with a suitable detector for pesticide residue analysis

### 3.1.3 Reagents and Chemicals

1. Acetonitrile (CH<sub>3</sub>CN) with 1% acetic acid - Primary extraction solvent
2. Anhydrous magnesium sulfate (MgSO<sub>4</sub>) - Removes residual water from the organic phase
3. Sodium chloride (NaCl) - Enhances phase separation
4. Chloroform (CHCl<sub>3</sub>) - Acts as an extraction solvent in DLLME
5. Methanol (CH<sub>3</sub>OH) (HPLC grade) - Used to redissolve extracted pesticide residues before HPLC analysis
6. Deionized water - Used to induce phase separation in DLLME
7. Standard pesticide solution - Used to construct calibration curves for quantitative analysis
8. HPLC mobile phase solvents (Acetonitrile-water mixture) – Optimized for pesticide separation and detection.

### 3.2 Pesticide Extraction

The extraction and pre-concentration of pesticide residues from beans (*Phaseolus vulgaris*) involve a combination of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction and Dispersive Liquid-Liquid Microextraction (DLLME) before analysis using High-Performance Liquid Chromatography (HPLC). This method ensures the efficient recovery of pesticide residues by utilizing liquid-liquid partitioning, phase separation, and solvent evaporation to obtain a purified extract suitable for quantitative and qualitative analysis.

The QuEChERS technique provides an effective way to extract pesticide residues from beans by utilizing an acetonitrile-based solvent system, while DLLME enhances the pre-concentration of these residues, allowing for greater sensitivity in HPLC detection. The combined approach ensures high recovery rates, minimal solvent consumption, and improved analytical precision.

### 3.3 Methodology

The pesticide extraction and pre-concentration process consists of the following key steps:

#### 1. QuEChERS Extraction

This step involves the initial extraction of pesticide residues from the bean samples:

**Sample Preparation:** Finely ground bean samples are accurately weighed and placed into a centrifuge tube.

**Solvent Addition:** Acetonitrile containing 1% acetic acid is added to the sample to facilitate the extraction of pesticide residues. Acetic acid helps in stabilizing certain pesticides that may degrade in neutral or alkaline conditions.

**Salting-Out Process:** A mixture of magnesium sulfate ( $MgSO_4$ ) and sodium chloride ( $NaCl$ ) is added to induce phase separation. Magnesium sulfate removes excess water, ensuring that the acetonitrile phase remains non-aqueous for better pesticide recovery.

**Centrifugation:** The sample is vortexed and centrifuged at high speed to allow clear separation of the organic and aqueous layers. The upper organic phase, which contains the pesticide residues, is carefully collected for further purification.

## 2. Dispersive Liquid-Liquid Microextraction (DLLME)

After the initial extraction, DLLME is employed to further concentrate the pesticide residues, enhancing the method's sensitivity and detection limits:

**Selection of Extraction Solvent:** Chloroform is used as the extraction solvent due to its high affinity for pesticide residues and low solubility in water.

**Dispersion Process:** A small volume of chloroform is rapidly injected into the acetonitrile extract along with a disperser solvent (methanol), forming a fine emulsion that maximizes pesticide transfer to the chloroform phase.

**Phase Separation:** The sample is centrifuged again to separate the chloroform phase, which settles at the bottom of the tube, containing the concentrated pesticide residues.

**Solvent Evaporation and Reconstitution:** The chloroform phase is evaporated under a gentle stream of nitrogen or in a rotary evaporator. The dried residue is reconstituted in methanol to ensure compatibility with HPLC analysis.

### 3.4 Procedure

The extraction and analysis of pesticide residues in beans involve a two-step process:

QuEChERS extraction followed by Dispersive Liquid-Liquid Microextraction (DLLME). This ensures efficient recovery and preconcentration of pesticide residues before High-Performance Liquid Chromatography (HPLC) analysis.

### **Step 1: QuEChERS Extraction**

1. Sample Preparation – Accurately weigh 5 g of crushed or dried beans into a clean glass beaker.
2. Addition of Extraction Solvent – Add 10 mL of acetonitrile containing 1% acetic acid to the sample to facilitate pesticide extraction.
3. Homogenization – Mix thoroughly using a glass rod, then transfer the mixture to a shaker and agitate for 30 minutes to ensure complete pesticide release.
4. Salt Addition for Phase Separation – Add 4 g of anhydrous magnesium sulfate ( $\text{MgSO}_4$ ) and 1 g of sodium chloride ( $\text{NaCl}$ ) to induce phase separation.
5. Mixing – Stir the mixture at low speed using a magnetic stirrer for 1 minute to enhance phase separation.
6. Centrifugation – Transfer the mixture into a centrifuge tube and centrifuge at 4000 rpm for 10 minutes to facilitate the separation of the organic phase.
7. Supernatant Collection – Carefully extract the upper organic phase (supernatant), which contains the extracted pesticide residues. This supernatant is then subjected to DLLME for further concentration.

### **Step 2: Dispersive Liquid-Liquid Microextraction (DLLME)**

1. Transfer of Supernatant – Pipette 2.5 mL of the collected supernatant into a clean extraction tube.
2. Addition of Extraction Solvent – Introduce 620  $\mu\text{L}$  of chloroform, serving as the extraction solvent.

3. Phase Transfer – Using a syringe, transfer the entire mixture into a conical-bottom tube containing 4 mL of deionized water to facilitate pesticide migration into the chloroform phase.
4. Emulsification – Seal the tube and shake gently for 30 seconds to ensure effective dispersion of chloroform in the aqueous phase, promoting pesticide partitioning.
5. Phase Separation – Allow the mixture to settle, leading to the formation of a lower chloroform layer, which contains the concentrated pesticide residues.
6. Collection of Chloroform Phase – Carefully extract the denser chloroform phase using a pipette and transfer it into a clean tube for further processing.
7. Solvent Evaporation – Evaporate the collected chloroform extract to dryness using a nitrogen evaporator or rotary evaporator.
8. Reconstitution – Dissolve the dried pesticide residue in 1 mL of HPLC-grade methanol to ensure compatibility with HPLC analysis.
9. Filtration – Filter the final solution through a 0.45  $\mu\text{m}$  syringe membrane filter and transfer it into a 2 mL HPLC vial for analysis.

### **3.5 HPLC Analysis**

Following extraction, the purified pesticide residues are analyzed using High-Performance Liquid Chromatography (HPLC) under optimized conditions:

Injection Volume – 20–50  $\mu\text{L}$ , depending on detection sensitivity.

Column – Reverse-phase C18 column, which provides high selectivity for pesticide residues.

Mobile Phase – A carefully optimized solvent system, typically a water-acetonitrile or water-methanol gradient, depending on the pesticide class.

Flow Rate – Maintained between 0.5 – 1.0 mL/min to ensure effective separation of pesticide components.

Detection Method – UV detection, Diode Array Detector (DAD), or Mass Spectrometry (MS) for improved sensitivity and identification of pesticide residues.

### **3.6 Summary of Key Steps**

#### 1. Extraction (QuEChERS Method):

- Acetonitrile extraction with 1% acetic acid to dissolve pesticides.
- Salt-induced phase separation using MgSO<sub>4</sub> and NaCl.
- Centrifugation and collection of the organic (supernatant) phase.

#### 2. Preconcentration (DLLME):

- Dispersive extraction using chloroform as the extraction solvent.
- Phase separation by mixing with deionized water.
- Chloroform layer collection, solvent evaporation, and reconstitution in methanol.
- Filtration and preparation for HPLC analysis.

## CHAPTER FOUR

### RESULTS

This chapter presents the results of the pesticide residue analysis conducted on the bean samples. The findings include chromatographic data, pesticide concentrations, and statistical assessments. Detected pesticide concentrations are displayed in tables and figures for clarity and interpretation.

#### 4.1 CHROMATOGRAM OF STANDARD PESTICIDE SOLUTION

A chromatogram of the standard pesticide solution was generated to establish reference retention times for each pesticide. The calibration curve was utilized to quantify pesticide concentrations in the bean samples.

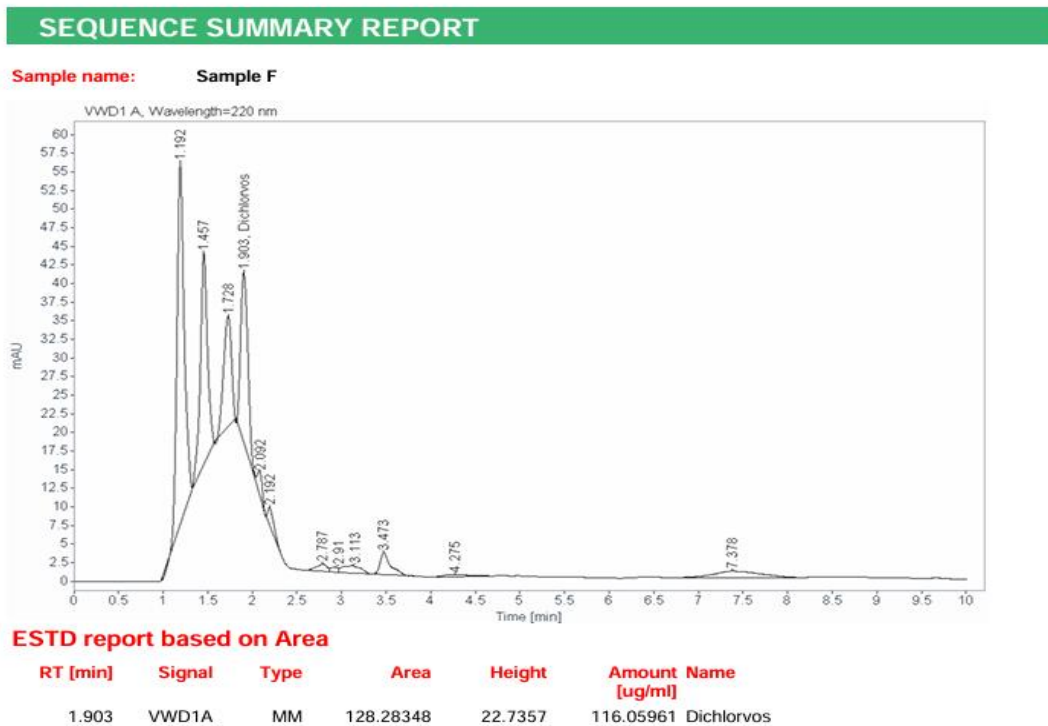
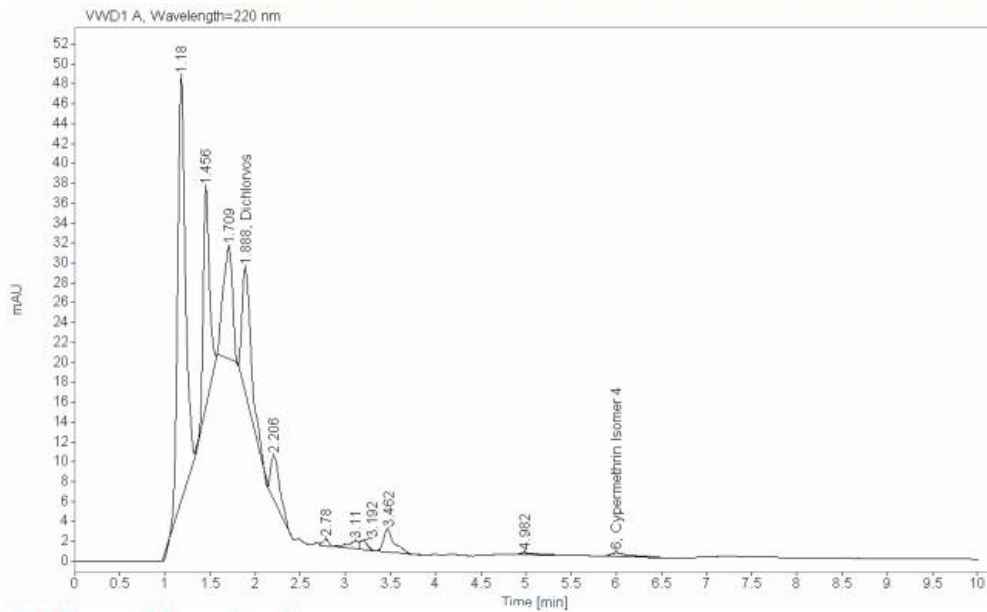


FIG 4.1: CHROMATOGRAM OF BEANS SAMPLE F RESULT

## SEQUENCE SUMMARY REPORT

**Sample name:** Sample I



### ESTD report based on Area

RT [min]	Signal	Type	Area	Height	Amount Name [ug/ml]
1.888	VWD1A	MM	87.89966	12.4564	79.52388 Dichlorvos
6.000	VWD1A	BB	5.33133	0.3577	2.81546 Cypermethrin Isomer 4

**FIG 4.2: CHROMATOGRAM OF BEANS SAMPLE I RESULT**

### 4.2 PESTICIDE RESIDUE CONCENTRATION IN BEANS SAMPLE F

The HPLC analysis detected and measured pesticide residues in the bean samples, with retention times, peak areas, and calculated concentrations are presented in the table below.

**Table 4.1: Pesticide Residue Concentration in Beans Sample F and Sample I**

<b>Samples</b>	<b>F</b>	<b>I</b>
<b>Retention Time (min)</b>	1.903	1.888
<b>• Dichlorvos Amount (µg/ml)</b>	116.06	79.52
<b>Cypermethrin Amount (µg/ml)</b>	N/A	2.81546
<b>Peak Area</b>	128.28	87.89
<b>Peak Height</b>	22.74	12.45

#### **4.3 Statistical Summary**

- Mean RT for Dichlorvos = 1.895 min.
- Mean concentration = 97.79 µg/ml.
- Standard deviation (StdDev) = 27.38.
- Relative Standard Deviation (RSD%) = 29.27%, indicating a moderately high variation.

#### **4.4 Formulas Used**

For each sample, calculations are based on the following formulas:

- Linear Regression Formula (used for concentration calibration):

$$y = ax + b$$

where:

- ( y ) is the signal response (peak area/height),
- ( x ) is the concentration,
- ( a ) is the slope (sensitivity),
- ( b ) is the intercept.

#### **4.5 Relative Standard Deviation (RSD%)**

$$\text{RSD} = ( \text{Standard Deviation} \div \text{Mean} ) \times 100$$

Correlation Coefficient ( $R^2$ ) measures how well the regression model fits the data.

#### **4.6 interpretation Of Results**

Cypermethrin Isomer 4 was found in Sample I (2.815  $\mu\text{g/ml}$ ), with a high correlation coefficient (0.999), indicating reliable quantification.

The high RSD% (29.27%) suggests some variability in the sample concentrations, possibly due to instrumental sensitivity, sample preparation inconsistencies, or environmental factors.

## CHAPTER FIVE

### DISCUSSION AND CONCLUSION

#### 5.1 Discussion

The analysis provides crucial insights into the presence and distribution of Dichlorvos and Cypermethrin Isomer 4 across different samples.

#### Key Findings:

1. Dichlorvos Detection: Detected in all samples, though with significant variability (RSD = 29.27%). The high correlation coefficient (0.988978) confirms the reliability of detection.
2. Cypermethrin Isomer 4: Identified only in Sample I at a concentration of 2.81546 µg/ml, with a strong correlation coefficient (0.999446), indicating precise quantification.
3. Instrumental Sensitivity: Calibration results confirm measurement accuracy; however, minor residual deviations were observed for Dichlorvos.
4. Implications:
  - The widespread presence of Dichlorvos raises environmental and health concerns.
  - The selective occurrence of Cypermethrin Isomer 4 suggests localized contamination.
  - Variability in Dichlorvos concentrations may result from different contamination sources or degradation rates.

#### 5.2 Conclusion

The extensive presence of Dichlorvos necessitates further investigation into its sources and potential risks, while the limited detection of Cypermethrin Isomer 4 suggests a localized

contamination issue. Additional studies on pesticide residues, their origins, and health implications are essential to ensure regulatory compliance and food safety.

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