HPLC ANALYSIS OF PESTICIDE RESIDUE IN *VIGNA UNGUICULATA* (COWPEA)

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, FACULTY OF BASIC MEDICAL SCIENCES UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR OF MEDICAL BIOCHEMISTRY (B.SC) DEGREE IN MEDICAL BIOCHEMISTRY

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CERTIFICATION

This is to certify that this project was successfully carried out by CHINKE JOSHUA CHINEMEREM with the matriculation number BMS2009087 , of the Department of Medical Biochemistry, School of Basic medical Sciences, University of Benin, Benin City.

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ABSTRACT

Cowpea (*Vigna unguiculata*) is a widely cultivated leguminous crop known for its high protein content, drought resistance, and economic importance, particularly in Africa, Asia, and Latin America. It serves as a major source of nutrition for millions of people, providing essential proteins, vitamins, and minerals. Cowpea production is significantly challenged by insect pests, including aphids (*Aphis craccivora*), thrips (*Megalurothrips sjostedti*), and pod borers (*Maruca vitrata*), which can cause substantial yield losses if not effectively controlled. To combat these pest infestations, farmers frequently use chemical pesticides to safeguard their crops and enhance productivity. However, excessive or improper pesticide application can lead to the accumulation of harmful residues in harvested cowpea, posing potential risks to both human health and the environment. For this study, cowpea (*Vigna unguiculata*) from different vendor were chosen to provide a broad analysis of pesticide residues. The cowpeas samples were categorized into eight sets, each consisting of raw samples and their corresponding milled forms. The raw samples were labeled and stored separately from the milled samples to ensure proper identification and traceability. High-Performance Liquid Chromatography (HPLC) was used for detection. The research followed a structured process, including sample collection, preparation, extraction, purification, and analysis using HPLC. The concentrations of the pesticides; Dichlorvos and Cypermethrin in the analyzed samples were calculated and expressed in µg/ml to indicate residue levels in the cowpeas. The concentrations of Dichlorvos in the analyzed cowpea samples varied: Sample E had the highest concentration (125.15 µg/mL), followed by sample F (116.06 µg/mL). Sample I had a moderate concentration (79.52 µg/mL). Sample G had the lowest concentration (52.73 µg/mL). Cypermethrin was only detected in sample I at a concentration of 2.82 µg/mL. The retention time (6.000 min) and peak area (5.33) confirm the presence of Cypermethrin but at a significantly lower concentration compared to Dichlorvos.These findings indicate that pesticide residue levels differ among samples, potentially due to varying pesticide application methods, time intervals between pesticide application and sample collection, or environmental degradation. The detected Dichlorvos concentrations (52.73 – 125.15 µg/mL) in this study significantly exceed the maximum residue limits (MRLs) set by international food safety authorities. Cypermethrin was detected in only one sample (2.82 µg/mL), which is significantly below the Codex Alimentarius MRL of 0.05 mg/kg (FAO/WHO, 2021). This study revealed high Dichlorvos concentrations in cowpea samples, exceeding permissible limits, while Cypermethrin levels were minimal. These findings highlight potential health risks and the need for stricter pesticide regulation and better agricultural practices.

CHAPTER ONE

1.1 Introduction

Cowpea (*Vigna unguiculata*) is a widely cultivated leguminous crop known for its high protein content, drought resistance, and economic importance, particularly in Africa, Asia, and Latin America (Singh *et al.,* 2020). It serves as a major source of nutrition for millions of people, providing essential proteins, vitamins, and minerals. Additionally, cowpea contributes to soil fertility by fixing atmospheric nitrogen, making it a valuable crop for sustainable agriculture (Tarawali *et al.,* 2021). Cowpea production is significantly challenged by insect pests, including aphids (*Aphis craccivora*), thrips (*Megalurothrips sjostedti*), and pod borers (*Maruca vitrata*), which can cause substantial yield losses if not effectively controlled (Ukeh *et al.,* 2019).

To combat these pest infestations, farmers frequently use chemical pesticides to safeguard their crops and enhance productivity. However, excessive or improper pesticide application can lead to the accumulation of harmful residues in harvested cowpea, posing potential risks to both human health and the environment (Odeyemi *et al.,* 2021). Studies have linked pesticide residues in food to various adverse health effects, including neurotoxicity, endocrine disruption, and carcinogenicity (Kumari *et al.,* 2022). Therefore, monitoring and regulating pesticide residues in cowpea are crucial to ensuring food safety and adherence to international standards established by organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (Jallow *et al.,* 2017).

High-performance liquid chromatography (HPLC) is one of the most effective analytical techniques for detecting and quantifying pesticide residues in food commodities. HPLC offers high sensitivity, accuracy, and reliability in residue analysis, making it a preferred method for food safety assessment (Patel *et al.,* 2020). This technique enables the separation and identification of multiple pesticide compounds within a sample, ensuring precise quantification of residues present in cowpea (Bajwa and Sandhu, 2014). The application of HPLC in pesticide residue analysis is crucial for determining compliance with established maximum residue limits (MRLs) and guiding policymakers in the implementation of safer agricultural practices (Kumari *et al.,* 2022).

Given the increasing concerns about food safety and environmental sustainability, it is imperative to conduct comprehensive studies on pesticide residues in cowpea. This study aims to evaluate the presence and concentration of pesticide residues in cowpea using HPLC, providing valuable insights into contamination levels and their potential health implications. The findings will contribute to food quality assurance, regulatory enforcement, and the promotion of responsible pesticide use in cowpea production.

1.2 Justification of the Study

Cowpea (*Vigna unguiculata*) is a widely cultivated legume, valued for its rich nutritional content and economic importance, particularly in tropical and subtropical regions (Singh *et al.,*2020). However, its productivity is significantly impacted by various insect pests, such as aphids (*Aphis craccivora*), thrips (*Megalurothrips sjostedti*), and pod borers (*Maruca vitrata*), necessitating the extensive use of pesticides for effective pest management (Ukeh *et al.,* 2019). While pesticides play a crucial role in ensuring higher yields, their indiscriminate and excessive application raises concerns about pesticide residues in cowpea, which may pose potential health risks to consumers and limit the crop's marketability due to strict regulatory standards (Odeyemi *et al.,* 2021).

High-performance liquid chromatography (HPLC) is recognized as a highly effective technique for detecting and quantifying pesticide residues in food products due to its high sensitivity, accuracy, and reproducibility (Jallow *et al.,* 2017). The application of HPLC in analyzing pesticide residues in cowpea is essential for assessing food safety, regulatory compliance, and potential health risks associated with pesticide contamination. By determining the levels and persistence of pesticide residues, this study will provide critical data to help policymakers establish appropriate maximum residue limits (MRLs) and promote the adoption of safer agricultural practices (Kumari *et al.,* 2022).

Given the increasing global emphasis on food safety and sustainable agricultural practices, this study is necessary to evaluate pesticide residues in cowpea using HPLC. The findings will not only contribute to food quality assurance but also raise awareness among farmers, consumers, and regulatory bodies about the implications of pesticide use in cowpea cultivation.

1.3 Aim of the Study

This study aim To assess the presence and concentration of pesticide residues in cowpea (Vigna unguiculata) using high-performance liquid chromatography (HPLC).Specific Objectives include:

- To determine the levels of pesticide residues in cowpea samples.
- To evaluate the compliance of detected pesticide residues with established maximum residue limits (MRLs).
- To assess the potential health risks associated with pesticide contamination in cowpea.
- To contribute to food safety monitoring and regulatory enforcement.
- To promote awareness and encourage the adoption of safer agricultural practices.

CHAPTER TWO

2.1 Literature review

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop used as food, cash crop and feedstuff for livestock. It has nitrogen fixing ability and aids in the prevention of hunger in Sub-Saharan Africa where food shortage is prevalent (Sariah, 2010). Cowpea is a popular legume which serves as a source of protein for many Nigerians who cannot afford animal protein (Animasaun *et al.,* 2015).

Figure 2.1: *Vigna unguiculata*

Source: Goncalves *et al.,* 2016).

One hundred grams of raw Cowpea grain contains 60g carbohydrate, 23.52g protein, minerals and vitamins (Affrifah *et al.,* 2021; Rangel *et al.,* 2003; Goncalves *et al.,* 2016). It also contains folic acid, lipids, essential and non-essential amino acids (Witthöft and Hefni, 2016). The northern parts of Nigeria with less rainfall are suited for cowpea production (Dugje *et al.,* 2009). Cowpea is either planted alone or as a companion crop when intercropped with cereals. The decay of its leaf litter, roots and root nodules produces nitrogen which enriches the soil

(Okereke *et al.,* 2006). Cowpea production is hampered by the presence of insect pests and diseases on the field and store (Omoigui *et al.*, 2018)

2.2 Varieties of cowpea and taxonomy

Cowpea is a *Dycatyledonea* belonging to the order *Fabales.* family *Fabaceae,* subfamily *Faboitleae,* tribe *Phaseoleae,* subtribe *Phaseolinae,* genus *Vigna,* and section *Catiang* (Verdcourt 1970; Marechal *et al.,* 1978). *Vigna* is a pantropical genus with several species, whose exact number varies according to authors: 184 (Phillips 1951), 170 (Paris 1965), between 170 and 150 (Summerfield and Roberts 1985), 150 (Verdcourt 1970), 54 (Sleele1976), and about 84 (of which some 50 species are indigenous to Africa) (Marechal *et al.,* 1978). In their revision of the genus *Vigna*, Marechal *et al*., (1978) subdivided the genus described earlier by Verdcourt (1970) into seven subgenera. In this classification, *V. unguiculata* (L.) Walpers and *V. nervosa* Markotter constitute the section *Catiang.* one of the six sections of the subgenus *Vigna.* Species of the section *Catiang* are characterized by spurred stipules below the attachment point of the leaf stalks and canoe-shaped keel with beak. The surface of their pollen grains are reticulate with raised exine (De Leonardis *et al*., 1993). Interspecific crosses made between the two species have not been successful (Mithen 1987; Ng and Apeji 1988; Ng 1995J. On the basis of a study on isoenzyme variation in the genera *Phaseohis* and *Vigna,* Jaaska and Jaaska (1988) proposed to raise the section *Catiang* to the rank of a subgenus.

All cultivated cowpeas are grouped under ***V. unguiculata*** subspecies ***unguiculata,*** which is subdivided into four cultigroups, namely Unguiculata, Biflora, Sesquipedalis. and Textilis (Westphal 1974; Marechal *et al.,*. 1978; Ng and Marechal 1985). There has been no major contention on this classification, since its adoption over 10 years ago.

The classification and nomenclature of the wild taxa within *V. unguiculata,* however, is complicated, and could sometimes be confusing. More than 20 epithet names have been used in the past to designate wild taxa within *V. unguiculata* species complex. An extensive work on characterization of over 400 wild *V. unguiculata* accessions was conducted at IITA (Ng and Padulosi 1991; Padulosi 1993). This work, coupled with surveys of live materials in the field and specimens in major herbaria in Europe and Africa, as well as cytological studies, has led to the description of new taxa, and a change of nomenclature of some species (Padulosi 1993; Ng 1995). Parallel work on taxonomy of wild species within section *Catiang* was also conducted elsewhere (Piennaar and Wyk 1992; Pasquet 1993).

The taxonomy of domesticated cowpea (*V. unguiculata* var. *unguiculata*) has a history of revisions, changes, and modifications that leave the nonexpert perplexed. The pantropical genus *Vigna* forms part of the subfamily Papilionoideae under the family Fabaceae (Leguminosae). Cowpeabelongs to the subgenus *Vigna*, section Catiang. It is genetically isolated from other *Vigna*, which includes only one other distinctly African species, bambara groundnut (*V. subterranea*). There are several Asian *Vigna* crop species such as urdcowpea (*V. mungo*), mothcowpea (*V. aconitifolia*), and mungcowpea (*V. radiata*). Morphological, ethnographical, molecular and other criteria led Pasquet (1999) to a classification of *V. unguiculata* that recognizes 11 subspecies, 10 of which are perennial and one of which (cowpea) is annual. Annual cowpea has two forms, the cultivated *V. unguiculata unguiculata* var. *unguiculata* and the wild/weedy form *V. u. u.* var. *spontanea*, both of which are inbreeding. *V. u. u. spontanea* is typically found only near the borders of cultivated cowpea fields and within them. The 10 perennial *V. unguiculata* subspecies include (i) some that are exclusively outcrossing: subspecies *baoulensis* (A. Chev.) Pasquet, ssp. *burundiensis* Pasquet, ssp. *letouzeyi* Pasquet, ssp. *aduensis* Pasquet, and ssp. *pawekiae* Pasquet, and (ii) others that are both outbreeding as well as inbreeding: ssp. *dekindtiana* (Harms) Verdc., ssp. *stenophylla* (E. Mey) Verdc., ssp. *tenuis* (E. Mey) Marechal, Mascherpa, and Stainier, ssp. *alba* (G. Don) Pasquet, and ssp. *pubescens* (R. Wilczek) Pasquet. Pasquet (personal communication to LLM) points out that the number of subspecies is likely to change as additional living material becomes available for study and as new molecular characterization tools are applied.

Originally only three, then later four cowpea cultigroups were recognized (Baudoin and Marechal, 1985). A fifth has recently been added (Pasquet, 1998). Smartt (1985) accounted for the emergence of two of the cultigroupson the basis of selection practiced in Asia after cowpea reached that continent, probably via India, about 2000 years ago. The cultigroups are: (1) Unguiculata, the African cowpea treated here, (2) Biflora, an erect woody perennial grown for fodder and seed, (3) Sesquipedalis, grown for its long, succulent pods in the Far East, (4) Textilis, cultivated in northern Nigeria and Niger; it has long peduncles, and is grown for the textile fibers it provides, (5) Melanopthalamus, originally from West Africa, is able to flower quickly under inductive conditions; the seeds have thin and often-wrinkled testa (Pasquet, 1998). The growth habit of cowpea ranges from indeterminate to determinate. As regards plant architecture, there is great variability. Plants range from erect, semi-erect, and prostrate (spreading, creeping) to climbing. One of the key features of cowpea is its long tap root, which enables the plant to obtain moisture at depths that cannot be reached by most plants.

The cowpea genome is estimated to be 613 Mb distributed amongst 22 chromosomes. In this regard, it closely resembles the model leguminous plant, *Medicago truncatula* (also estimated at 613 Mb) (Arumuganathan and Earle, 1991). The sequencing and analysis of whole genomes of model plants, such as *Arabidopsis thaliana*, have paved the way for orphan crops like cowpea,where far fewer resources are available for research (Mahalakshmi and Ortiz, 2001).

NUTRITIONAL VALUE OF COWPEA (*Vigna uguiculata*)

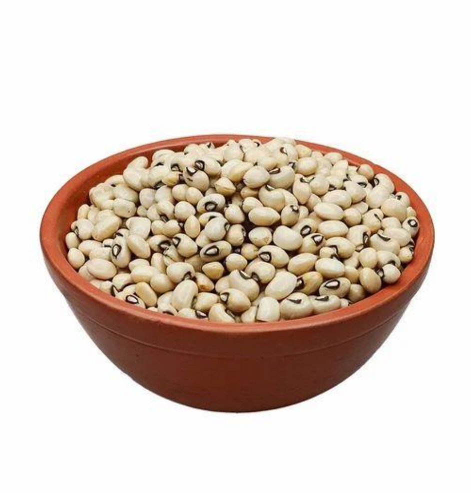


Figure 2.2: *Vigna unguiculata*

Source: Anderson *et al.,* 2009).

Cowpea (*Vigna unguiculata*) a nutrient-dense food, providing essential macronutrients, micronutrients, and beneficial bioactive compounds. With a protein content of 20–25% on a dry weight basis, cowpea play a vital role in vegetarian diets and regions where animal proteins are scarce. While not a complete protein on their own, cowpea complement grains to provide all essential amino acids when consumed together (Messina, 2014; Leterme, 2002). Their rich fiber and complex carbohydrate content result in a low glycemic index, making cowpeas beneficial for blood sugar regulation, cardiovascular health, and digestion (Anderson *et al.,* 2009).

Cowpea also serve as a valuable source of micronutrients. They contain significant amounts of iron, which supports oxygen transport, and folate, which is essential for DNA synthesis and prenatal health (Mitchell *et al.,* 2016). Additionally, cowpea provide magnesium and potassium, both of which contribute to strong bones, fluid balance, and blood pressure control (Karanja *et al.,* 2014). Their B vitamin content aids in energy metabolism, while their antioxidants—such as flavonoids and polyphenols—help combat oxidative stress and reduce the risk of chronic diseases (Beninger and Hosfield, 2003). The fiber in cowpea not only promotes gut health and satiety but also assists in cholesterol management.

Regular cowpea consumption has been linked to a reduced risk of cardiovascular disease, type 2 diabetes, and certain cancers, highlighting its significance as a functional food (Guillon and Champ, 2002). In sub-Saharan Africa, particularly in rural areas, cowpea are instrumental in addressing protein malnutrition. For instance, in Uganda, where access to meat is often limited, smallholder farmers rely on cowpea as a primary protein source. A 2018 study by Mutyaba *et al.*, found that cowpeas accounted for 45% of the protein intake in rural Ugandan households, especially during dry seasons when other protein sources are scarce. This underscores the crucial role cowpea play in food security and nutrition, particularly in resource-constrained settings.

2.2 Storage of cowpea

Proper cowpea storage is crucial for maintaining their nutritional quality, safeguarding them from pests, and minimizing post-harvest losses. Various storage techniques, both conventional and modern, are employed based on available resources, technological advancements, and local agricultural practices. These methods differ in their efficiency, cost, and suitability for prolonged storage.

2.2.1 Traditional storage methods

Traditional methods of storing cowpea play a significant role in many agricultural communities, particularly in developing regions where access to advanced storage technologies is limited. These approaches aim to prevent spoilage, protect cowpeas from pests, and shield them from environmental conditions using locally available materials. Some of the most commonly practiced techniques include storing cowpeas in woven sacks, clay vessels, and underground storage pits. These methods, while effective to some extent, offer varying levels of success in preserving cowpeas for extended periods.

One prevalent method involves the use of woven bags, commonly crafted from materials such as jute, sisal, or polypropylene. These bags are favored due to their affordability, lightweight nature, and ease of transportation, making them particularly beneficial for smallholder farmers and rural dwellers (Adejumo and Raji, 2007). Despite these advantages, woven bags offer minimal defense against moisture and temperature variations. In areas with high humidity, cowpeas stored in these bags are prone to absorbing moisture, which increases the likelihood of mold formation and deterioration in quality. Additionally, woven bags are not pest-proof, as insects can easily infiltrate them, causing infestations that lead to a reduction in both quantity and nutritional value (Chikoye *et al.*, 2015).

Another traditional storage method involves the use of clay pots, a technique commonly adopted in arid regions. Clay pots are particularly valued for their ability to maintain a stable internal temperature, which helps protect stored cowpea from excessive heat. The porous texture of clay facilitates slight air exchange, thereby reducing the accumulation of moisture and, to some extent, mitigating mold growth. However, clay pots present certain drawbacks, including limited storage capacity and susceptibility to cracking or breakage. Furthermore, while they offer a moderate level of pest resistance, they do not provide complete protection against insects, particularly when cracks develop in the structure over time (Kimatu *et al.*,2012).

In some cultures, underground pits or silos are utilized as storage facilities for cowpea. This ancient practice is known for its effectiveness in maintaining grain quality by providing a cool, stable environment that minimizes exposure to fluctuating temperatures. In dry regions, lining pits with straw or ash can create insulation and deter pest infestations. However, this method is not without challenges. In humid environments, underground storage can lead to excessive moisture retention, accelerating spoilage. Additionally, the process of digging and preparing pits requires significant labor, and frequent access to stored cowpeas can be inconvenient (Murdock *et al.,* 2003).

Although these traditional storage practices are widely used due to their affordability and accessibility, they often fail to regulate key environmental factors such as temperature, humidity, and pest infestations, all of which are critical for optimal cowpea preservation. In tropical and subtropical areas, high humidity levels pose a significant threat to traditional storage systems, as moisture accumulation fosters mold and fungal growth, ultimately compromising the quality and safety of cowpea. The absence of modern pest control measures further exacerbates the problem, leading to the risk of contamination with harmful mycotoxins, which pose health hazards to consumers (Bankole and Adebanjo, 2003).

Despite their limitations, traditional storage techniques continue to be widely utilized in many rural communities due to their low cost and ease of implementation. However, they often struggle to maintain cowpea quality over long periods, particularly in humid environments where moisture control remains a persistent challenge.

2.2.2 Modern storage methods

To overcome the drawbacks of traditional storage, modern storage techniques have been developed to enhance the preservation of cowpeas by controlling factors such as temperature, humidity, and pest infestations. These advanced approaches utilize technology to create optimal storage environments, thereby extending shelf life and ensuring high-quality, safe cowpea for consumption.

One of the most effective modern storage techniques is hermetic storage, which involves the use of airtight containers, typically made from polyethylene or metal, to create a sealed environment. By eliminating external air circulation, hermetic storage deprives pests and microorganisms of oxygen, preventing their survival and subsequent contamination of the cowpea. This technique effectively curtails the growth of fungi and insect infestations without relying on chemical pesticides, thereby ensuring longer preservation of cowpea (Baoua *et al.*,., 2012). A study conducted by the International Center of Insect Physiology and Ecology (ICIPE) in 2019 found that hermetic storage reduced post-harvest losses by up to 70% over six months. This highlights the effectiveness of this storage method in maintaining cowpea quality while minimizing dependency on chemical-based pest control measures.

Another highly efficient modern storage approach is cold storage, which involves keeping cowpea at consistently low temperatures to slow down metabolic activities in pests and microorganisms, thereby preventing spoilage and infestation. Cold storage is particularly useful for long-term preservation and is commonly employed in commercial warehouses and large-scale agricultural enterprises. This method is highly effective in maintaining the nutritional integrity and sensory characteristics of cowpeas, making it ideal for premium-quality or export-grade cowpeas (Tefera *et al.*, 2011).

However, despite its benefits, cold storage has certain limitations. It requires substantial energy inputs and specialized infrastructure, making it an expensive option that is often inaccessible to small-scale farmers, particularly in areas with unreliable electricity supply. The high operational costs associated with refrigeration and temperature regulation make this method more feasible for industrial-scale agricultural operations rather than smallholder farmers.

While modern storage techniques offer superior protection against moisture, pests, and temperature fluctuations, their widespread adoption is often hindered by economic and infrastructural constraints. Nevertheless, these methods provide highly effective solutions for maintaining the quality and longevity of stored cowpeas, ensuring food security, and reducing post-harvest losses.

Modified Atmosphere Packaging (MAP) has become an increasingly popular technique for maintaining the quality of cowpea during storage and transportation. This method involves altering the concentrations of oxygen, carbon dioxide, and nitrogen inside sealed packaging to create an environment that inhibits pest activity and microbial growth. By elevating carbon dioxide levels while reducing oxygen, MAP lowers the respiration rates of both cowpea and pests, effectively slowing spoilage (Mutungi *et al.,* 2012). Research has shown that MAP can significantly prolong the shelf life of cowpea while preserving their nutritional value, color, and flavor. Despite its effectiveness, implementing MAP requires specialized technology and monitoring systems, making it a costly option that may not be viable for smallholder farmers (Mutungi *et al.,* 2012).

A similar approach to MAP is **vacuum sealing**, which removes air from the packaging before sealing it. This process creates a low-oxygen environment akin to hermetic storage, without the need for a specialized container. Vacuum sealing is particularly effective in reducing both moisture and oxygen levels, thereby preventing the growth of aerobic microorganisms. Studies indicate that this technique can enhance the longevity of stored cowpeas, helping to maintain their nutritional content and prevent deterioration (Mahajan *et al.*, 2014). However, as with MAP, vacuum sealing necessitates the use of specialized equipment, which can be expensive and inaccessible to small-scale farmers.

In addition to these packaging technologies, **chemical treatments** and protective **coatings** are also employed to improve cowpea storage. These methods involve applying insecticides, fungicides, or protective substances directly onto cowpeas or their storage containers to safeguard against pests and microbial contamination. Chemical treatments have proven effective in preventing infestations and spoilage, thereby extending storage duration (Ukeh *et al.,*., 2012). However, concerns regarding chemical residues and potential health risks have limited their widespread use. Moreover, these treatments often require trained personnel and strict adherence to safety regulations, making them less practical for smallholder farmers.

While these storage methods offer significant advantages in preserving cowpea quality and extending shelf life, they also come with challenges. The reliance on specialized equipment, energy consumption, and high initial costs can make them inaccessible for farmers with limited resources. Nevertheless, for commercial agricultural enterprises and large-scale operations—especially those involved in export markets—these techniques play a crucial role in ensuring long-term cowpea storage and maintaining high-quality standards.

2.3 Economic importance of cowpea

Cowpea is today grown throughout the world, with the most intense production in the northern savannahs of sub-Saharan Africa, with Nigeria and Niger the leading producers. According to Langyintuo *et al.,* (2003), some 10 million hectares are under cowpea cultivation worldwide, with the sub-Saharan Africa cowpea belt producing about two-thirds of the annual world yield. Total annual grain production is about 3.7 million tons. The second largest production area after Africa is Brazil, where the crop is well suited to the relatively low rainfall and poor soils in the northeastern part of the country. Cowpea is also grown in marginal areas of eastern and southern Africa, especially in Sudan, Somalia, Mozambique, Botswana, and southern Zimbabwe. Cowpea is mostly grown as an intercrop with cereals, but little of that harvest reaches regional markets. The most important export market for cowpea in West Africa is Nigeria, simultaneously the world’s largest cowpea consumer as well as producer. There is significant cowpea production in the Mediterranean, South Asia, and in the southern and southwestern United States Total world harvested area of cowpea in the

1990s was 9 738 000 ha, 7 804 000 of which was produced in only 12 nations in West and Central Africa. The greatest production (1 691 000 tons) was in Nigeria, followed by its neighbor to the north, Niger, with 359 000 tons (Langyintuo *et al.*,2003). The importance of cowpea as a nutritional complement to the mostly cereal and root-andtuber-based diets of West and Central Africa has resulted in an ancient cowpea trade system. Demand for cowpea is particularly high in Nigeria. Its dense population and large oil revenues create enormous effective cowpea demand by both urban and rural consumers. Cowpea markets in West Africa link the humid coastal zones with the semi-arid interior. In essence, cowpea produced in the drier regions (northern Nigeria, Niger, Burkina Faso, northern Ghana, northern Cameroon, etc.) flows southward toward or into Nigeria, the most populous of the African nations. This pattern of cowpea trade toward the Gulf of Guinea has been given the name “Nigerian Grain Shed”. A second, smaller trading geography is found to the west. The Senegalese Grain Shed occupies Senegal, Mauritania, and parts of Mali. In West and Central Africa, grain marketing is organized through formal and informal channels. Formal market places are subject to government control. Informal markets are not officially recognized but still operate well, though outside of government control. An informal market may be nothing more than a group of women or men who meet to carry out a transaction in a village or at a roadside. Farmers who are constrained by liquidity or transportation may accept quick transactions and lower prices compared to the generally prevailing market prices. Farmers usually sell their cowpeas to rural assemblers, who in turn sell them to urban wholesalers directly, or through intermediaries. Wholesalers sell large stocks when prices are high enough to pay for transaction costs (procurement, storage, and handling) plus a profit margin. They may also be involved in trade of other commodities, such as cereals and groundnut. There is no reliable source of year-to-year cowpea production or marketing data in Africa. Information on cowpea marketing and trade is scarce and data on cowpea production economics are scattered, because marketing research has focused on African export crops such as cocoa, coffee, cotton, and groundnut and to a lesser extent cereals (Van der Laan cited by Langyintuo *et al.,.*, 2003). In West Africa, protein products traditionally move south from the drier areas to the more equatorial humid areas, while carbohydrate products move north. Thus, it is that cowpea is also traded from West to Central Africa because of the comparative advantage that the drierareas of West Africa have in protein production—cowpea being a major source of protein (Langyintuo *et al.,.*, 2003). At least 285 000 tons of cowpea are shipped among countries in the region each year. This is probably an underestimate because the official sources for this estimate did not collect data on all formal and informal flows of grain. In 1998, Burkina Faso imported about 8000 tons from Niger and exported a total of 5500 tons to Togo, Cote d'Ivoire, Ghana, and Benin. It is estimated ˆ that Nigeria's average annual imports of 260 000 tons per year from Niger accounts for about 73% of Niger's surplus production. Cowpea trade between Nigeria and Benin is bilateral. Togo and Ghana, and Ghana and Benin trade bilaterally as well. Gabon depends on Cameroon, Togo, Benin, and Nigeria for cowpea. Mauritania, Gambia, and Guinea Bissau rely on Senegal (Langyintuo *et al.*, 2003; INPhO (FAO), 2007).

2.4 Diseases of cowpea

Cowpea is susceptible to diseases that affect legumes. The fungal diseases include: Damping-off (*Pythium* spp.) may occur on seedlings under moist conditions and in dense plantings. Root rot (*Verticillium* spp.) and stem rot (*Fusarium* spp.) may also be a problem. Cowpea is susceptible to powdery mildew (*Erysiphe polyqoni*) during wet winter months and under humid conditions. Other diseases that affect cowpea include anthracnose (*Colletotrichum lindemuthianum)*, charcoal rot (*Sclerotium bataticola*), and fusarium wilt (*Fusarium oxysporum vr. tracheiphilum).* Viral diseases include: Cowpea aphid borne mosaic virus

(CABMV) Genus potyvirus, blackeye cowpea virus (BLCMV) Genus potyvirus, cowpea mosaic virus (CPMV) Genus comovirus, cowpea mottle virus (CPMOV) Genus carmovirus.

Nematodal diseases include: Root knot nematode (*Meloidogyne* spp.), root lesion nematode (Pratylenchus spp.), dagger nematode (*Xiphinema* spp.). While bacterial diseases include: Cowpea blight (*Xanthomonas campestris pv. Vignicola*), cowpea bacterial pustule *(Xanthomonas campestris pv. Vigna unguiculata*). In the establishment phase, rodents and birds can be important pests by feeding on the seeds.

Cowpea production is threatened by various diseases caused by fungi, bacteria, and viruses, leading to significant yield losses (Emechebe and Florini, 1997).

Major Diseases of Cowpea

A. Fungal Diseases

1. Anthracnose (Colletotrichum lindemuthianum)

Colletothrichum sp. induces two major diseases in cowpea (anthracnose and brown blotch) in the humid forest of South-western Nigeria. These diseases are induced by two different species of the genus *Colletotrichum*. Emechebe and Florini (1997) had suggested that the cowpea anthracnose pathogen be regarded as a species that is distinct from *Colletotrichum lindemuthianum*, the *Phaseolus* cowpea anthracnose pathogen. (Latunde-Dada *et al.,* 1999) have provided strong evidence in favour of considering the cowpea anthracnose pathogen as a form of *Colletotrichum destructivum* O'Gara and this has been accepted and adopted (Allen and Lenne, 1998). In Savannah agro-ecologies of Nigeria, cowpea brown blotch disease is induced by *Colletotrichum capsici* (Allen and Lenne, 1998; Emechebe and Shoyinka, 1985).

However, *Colletotrichum truncatum* (Schew) Andrus and More is regarded as the causal agent of brown blotch of cowpea in humid forest of South-western Nigeria (Adebitan, 1984). Symptoms of the disease includes purplish brown discolouration on pods, which may also extend to petioles, leaf veins and peduncles. Pod infection often leads to maldevelopment and distortion of pods (Allen *et al.,* 1998). The diseases have been found to be seed borne (Emechebe and McDonald, 1979). The role of toxic metabolites of the pathogens inducing both the anthracnose and brown blotch disease development and symptoms manifestation in cowpea in the humid forest of southern Nigeria has been demonstrated (Amusa, 1991).

Symptoms: Dark, sunken lesions on stems, pods, and leaves, often leading to defoliation.

Causes: Spread by infected seeds and humid conditions (Adegbite and Amusa, 2008).

Management: Use of resistant varieties, fungicide application, and crop rotation. Causes: Spread by infected seeds and humid conditions (Adegbite and Amusa, 2008).

2. Cercospora Leaf Spot (*Cercospora spp*.)

Cercospora leaf spot is induced by *Cercospora canescens* Ellis and Martin, while Pseudocercospora leaf spot is induced by *Mycosphaerella cruenta* Latham in the form of its anamorph, *Pseudocercospora cruenta* (Sacc.) Deighton (formerly *C. cruenta)* (Allen and Lenne, 1998). Pseudocercospora leaf spot is characterized by chlorotic or necrotic spots on the upper leaf surface and profuse masses of conidiophores and conidia, appearing as downy gray to black mats, on the lower leaf surface. *Cercospora* leaf spot is characterized mostly by circular to irregular cherry red to reddish-brown lesions on both leaf surfaces.

Both pathogens survive the no-crop period on infected crop residue and in infected seed (Williams, 1975; Scheneider *et al.,* 1976). *P. cruenta* induces leaf spot on several legumes and *C. canescens* on an even wider range of legumes (Emechebe and McDonald, 1979).

However, Pseudocercospora leaf spot is economically more important than *Cercospora* leaf spot. Out of 75 cowpea lines evaluated in 1999 and 2000, about 40% of the germplasm was found susceptible to *cercospora* leaf spot diseases (Ajibade and Amusa, 2001). Ife brown, a widely adopted and cultivated cowpea cultivars in Southwestern Nigeria had 80% *cercospora* incidence on the field. Field observation revealed crop loss of over 40% in *cercospora* endemic field.

Symptoms: Small, circular brown or reddish spots on leaves, which may cause premature defoliation.

Causes: Airborne fungal spores and prolonged humidity (Owolade *et al.*, 2005).

Management: Use of disease-free seeds, proper plant spacing, and application of fungicides.

3. Powdery Mildew (*Erysiphe polygoni*):

Cowpea powdery mildew is induced by the oidial phase (Oidium spp.) of Erysiphe polygon! DC and Sphaerotheca fuiiginea. E. polygoni is prevalent in all cowpea growing regions, but S. fuiiginea has been reported only from India (Jhooty *et al.*,1985).

The diagnostic sign of this disease is copious, white, powdery fungal growth, mainly consisting of oi'dia, the repeating spores of the fungus, on the upper leaf surface. Chlorotic and then brown patches appear first on the undersurface of the leaf, and they later become distinct on the upper leaf surface. Severely mildewed leaflets fall, resulting in partial or complete defoliation of the plant.

E. polygoni has a broad host range of more than 500 species of higher plants, both annuals and perennials, especially in the family Leguminosae (Ainsworth 1971). The fungus probably perpetuates itself on these hosts from one season to another as conidia; ascospores have not been detected in the tropics. Disease development in Latin America and Zambia was favored by wet weather (Lin and Rios 1985; Kannaiyan *et al.*,.1987). By contrast, in the Sudan savanna zone of Nigeria, we observed moderate damage by powdery mildew during the dry period at the end of the rainfed season and greater severity in irrigated, dry-season cowpea than in rainfed cowpea of the same variety. The disease is also destructive under hot, dry conditions in the screenhouse. In India, the disease increases rapidly during the dry and cool season (Mew et a). 1985). Since there are several races of the pathogen (Lin and Rios 1985), it is reasonable to expect the differences in the above reports. Indeed, Rodriguez and Melendez (1984) have suggested that there is a new race capable of attacking cowpea under high relative humidity and heavy rains in Puerto Rico.

Cowpea powdery mildew is important in Zambia (Kannaiyan *et al.,* 1987), Zimbabwe (Mariga *et al.,* 1985), Florida, USA (Stoffella *et al.,* 1990), Puerto Rico, and other cowpea producing countries of Latin America (Rodriguez and Melendez 1984; Lin and Rios 1985).

The disease is so important in India that fungicidal sprays have been recommended for its control (Singh and Anilkumar 1986). However, we found no estimates of yield losses due to powdery mildew in cowpea.

Two control methods have received the greatest attention: growing resistant varieties and application of fungicides. (Lin and Rios 1985) noted that resistant cultivars exist in Latin America but their use is limited by the occurrence of races, presumably with matching virulence genes. In India, both highly resistant and partially resistant lines have been identified (Raju and Anilkumar 1990, 1991). In Zambia, Kannaiyan *et al.,* (1987) found no line to be resistant out of 140 entries, although two of them were moderately resistant (scoring 2-3 on a rating scale of 1-9). Fungicides have been evaluated as seed, soil, or foliar treatments for the control of cowpea powdery mildew. Singh and Anilkumar (1986) concluded that effective protection of cowpea was obtained by seed treatment with carbendazim, followed by one foliar-applied spray of triadimefon. In Puerto Rico, Rodriguez and Melendez (1984) obtained very effective control of powdery mildew with dinocap in the dry season but not in the rainy season.

Biweekly application of 0.26 kg/ha of benomyl also protected cowpea from infection by E. polygoni.

Symptoms: White powdery growth on leaves and stems, leading to leaf yellowing and defoliation.

Causes: Dry weather with high humidity (Emechebe & Florini, 1997).

Management: Planting resistant varieties, improving air circulation, and applying sulfur-based fungicides.

B. Bacterial Diseases

1. Bacterial Blight (*Xanthomonas axonopodis pv. vignicola*):

Bacterial blight and bacterial pustule. Bacterial blight (induced by *Xanihomonascampestris pv. vignicola* [Burkholder] Dye) is probably the most widespread disease of cowpea, having been reported from all regions of the world in which cowpea is cultivated.

By contrast, bacterial pustule has a more restricted distribution; until the recent report of its occurrence in Nepal by (Dahal *et al.*, 1992), it was considered to be limited to Africa (Patel 1981). There is slill some controversy about the species of Xanthomonas that induces bacterial pustule. Based on differences in pathogenic behavior of the bacterial blight and the pustule pathogens, (Patel and Jindal 1982) suggested that the pustule pathogen should be regarded as a distinct pathovar of X. campestris, namely *X. campesrris pv. vignaeimguiculatae*. Emechebe and Shoyinka (1985) speculated that it could be a strain of the bacterial blight pathogen. X. campesrris pv. vignicola, and preliminary characterization of 120 isolates from pustule or blight symptoms support their point of view (K. Wydra.personal communication, HTA. Cotonou, Benin). Pathogenic variability has been reported for both pathogens; Pate] (1981) reported the existence of three races of the bacterial pustule pathogen, while Prakash and Shivashanker (1982) suggested that the race of the bacterial blight pathogen prevalent in India differs from that prevalent in Nigeria.

The pathogens of both diseases are seed transmitted, while secondary spread occurs by wind-driven rain (Preston 1949; COPR 1981). Insects have also been implicated in secondary spread of the bacterial blight pathogen (Kaiser and Vakili 1978). Both diseases cause premature leaf fall and water-soaked dots on the undersurface of leaves (Williams 1976). Unlike bacterial pustule, bacterial blight induces large, irregular foliar lesions with yellow margins (Palel 1982), stem cankers, and both preemergence and postemergence seedling mortality (Kishun 1989).

Total crop loss in susceptible varieties may result from seedling cankers or severe cankers of peduncles and floral cushions on older plants. Kishun (1989) working in India—where bacterial blight is considered the most destructive among all cowpea diseases (Prakash and Shivashanker 1982)—reported grain yield losses of 2.7-92.2%. depending on the susceptibility of the variety.

Apart from the work of Ekpo (1978, quoted by Allen 1983), who reported yield losses due to bacterial pustule of 1.8% and 26.6% in resistant and susceptible varieties, respectively, the only other attempt to quantify losses caused by bacterial pustule was that of Omotunde (1987) at Ibadan. Nigeria. He reported 76.8% and 2.3% losses in grain yields of susceptible (TVx 301) and resistant (TVu 43) lines, respectively.

The influence of some cultural practices on the severity of bacterial blight has received relatively little attention. Rao and Hi remain (1985) in India showed that disease severity was increased by N and P applications, but was decreased by the applications of moderate levels of K and Mo. and high doses of Ca and Mg. In Kenya. Ouko and Buruchara (1987) showed the contrasting effects of cropping system on the incidence and severity of bacterial blight in cowpea grown in the long or the short rainy season. At 40 days after inoculation during short seasons, disease incidence was 62.5% in a cowpea/maize intercrop, compared to 75% in a sole crop of cowpea and 92.3% in a cowpea-maize relay crop. By contrast, in long seasons, blight incidence was 68.7% in a cowpea-maize relay crop and 100% in both sole cropped cowpea and a cowpea/maize intercrop. In a sowing date trial in India, Kishun and Chand (1989) showed that damage by bacterial blight was lower in an early-sown crop than in a later-sown crop, and that the disease intensified with an increase in plant populations.

(Emechebe and Shoyinka 1985) suggested that the incidence and severity of both diseases would decrease if farmers sowed 6nly pathogen-free seeds. Soni and Thind (1991) showed that it was easy to obtain pathogen-free seeds from healthy pods. The effectiveness of this control measure can be enhanced by seed treatment with an antibiotic or a mixture of an antibiotic and a fungicide, such as streptocycline (100 u.g/ml) plus captan (2000 ug/ml) (Jindal and Thind 1990). Suitable rotations of three consecutive cowpea-free growing seasons should also be effective against these host-specific xanthomonads.

Symptoms: Water-soaked lesions on leaves that turn brown and spread, often affecting pods.

Causes: Spread through infected seeds, rain splashes, and contaminated tools (Emechebe, 1988).

Management: Use of certified seeds, avoiding overhead irrigation, and practicing crop rotation.

C. Viral Diseases

1. Cowpea Mosaic Virus (CPMV):

CPMV is a non-enveloped virus belonging to the Comovirus genus. It primarily infects cowpea plants, causing symptoms like mosaic patterns on leaves, which can lead to reduced photosynthesis and stunted growth. The virus is transmitted by certain beetle species, including the cowpea weevil (*Callosobruchus maculatus*), which can cause significant yield losses if not managed properly.

Symptoms: Mosaic patterns on leaves, stunted growth, and poor pod development.

Causes: Transmitted by aphids and infected seeds (Sharma *et al.,* 2015).

Management: Controlling aphids using insecticides, planting virus-resistant varieties, and removing infected plants.

2. Cowpea Aphid-Borne Mosaic Virus (CABMV):

CABMV is a member of the Potyvirus genus and is predominantly transmitted by aphids, especially *Aphis craccivora*, in a non-persistent manner. Infected plants exhibit symptoms such as mosaic patterns, leaf malformation, and overall plant stunting. The virus can also be seed-transmitted, with a transmission rate of approximately 5%, facilitating its spread across regions.

Symptoms: Yellow mosaic, leaf distortion, and reduced pod production.

Causes: Aphid transmission from infected plants (Thottappilly & Rossel, 1993).

Management: Use of resistant varieties, aphid control, and reflective mulches to repel vectors.

2.5 Pest of cowpea

Insects are the major causes of crop losses in cowpea. In some years and areas, grain yields can be reduced to nearly zero, if the crop is not sprayed with insecticide. Aphids, flower thrips, legume pod borers (*Maruca vitrata*), and a complex of pod sucking bugs each and collectively cause yield losses. In northern Nigeria, for example, untreated cowpea plots yielded 76 kg ha−1, while fields treated with the carbamate insecticide Carbaryl (at 1.12 kg ha−1) yielded

1382 kg ha−1. Similar yield increases were seen after treatment with chlorinated hydrocarbons (Raheja, 1976).

The insect problems of cowpea are not limited to the field. When the harvested grain is subsequently stored, beetles of the family Bruchidae (*Callosobruchus maculatus*) cause major losses.

The insect problems of cowpea are compounded by the fact that chemical insecticides not officially approved for cowpeas (e.g., cotton insecticides) are often used on them anyway, both in the field as well as in storage.

Cowpea (*Vigna unguiculata*) cultivation is significantly affected by various insect pests, leading to substantial yield losses. Below is an overview of notable pests and relevant studies addressing their management:

1. Legume Pod Borer *(Maruca vitrata*):

Maruca vitrata is a major pest targeting the flowers and pods of cowpea, causing yield reductions ranging from 20% to 80%. The larvae feed on flower buds, flowers, and young pods, compromising both quantity and quality of the produce (Sharma, H.C. 1998).

2. Cowpea Weevil (*Callosobruchus maculatus*):

This beetle is a notorious storage pest, infesting cowpea seeds during storage and causing significant post-harvest losses. The larvae develop inside the seeds, rendering them unfit for consumption or planting (Lienard, *et al.*, 1993).

3. Cowpea Aphid (*Aphis craccivora*):

This aphid species attacks cowpea by sucking sap from leaves, stems, and pods, leading to stunted growth and reduced yields. They also act as vectors for viral diseases (Jackai., *et al.*, 1986).

4. Flower Thrips (*Megalurothrips sjostedti*):

These tiny insects feed on cowpea flowers, causing flower abortion and pod malformation, which directly impacts yield (Ekesi *et al.,* 2000).

5. Cowpea Curculio (*Chalcodermus aeneus*):

This weevil infests cowpea pods, leading to seed damage and potential yield losses (Capinera, J.L. (2002).

Effective management of these pests involves integrated pest management (IPM) strategies, including cultural practices, biological control agents, resistant cowpea varieties, and judicious use of chemical pesticides.

2.5 PESTICIDE

Pesticides are chemical or biological substances used to control pests, including insects, weeds, fungi, and rodents, to enhance agricultural productivity and prevent disease transmission (Aktar *et al.,*., 2009). These substances can be synthetic or derived from natural sources and are categorized based on their target organisms and chemical structure.

2.5.1 Importance of pesticides in agriculture

Pesticides help improve crop yield, reduce post-harvest losses, and minimize the spread of plant and animal diseases. However, their misuse or overuse can lead to environmental pollution, pest resistance, and health hazards (Pimentel *et al.,* 2005).

2.5.2 Types of pesticides

Pesticides are classified into different categories based on their target organisms, chemical composition, and function.

Classification based on target organism

Insecticides

Insecticides are chemical or biological substances used to kill, repel, or control insect populations that threaten crops, livestock, and human health. These substances are widely used in agriculture, public health, and household pest control (Ware & Whitacre, 2004).

Insects can cause severe damage to crops by feeding on leaves, stems, and roots, reducing agricultural productivity. Additionally, some insects act as vectors for diseases like malaria, dengue fever, and Zika virus, making insecticides a critical tool in disease prevention (Matthews, 2018). Common types of insecticides include:

Organophosphates (e.g., Malathion, Chlorpyrifos): Affect the nervous system by inhibiting acetylcholinesterase enzymes (Casida & Durkin, 2013).

Pyrethroids (e.g., Permethrin, Cypermethrin): Synthetic versions of natural pyrethrins from chrysanthemum flowers, commonly used in household insect control (Davies *et al.*, 2007).

Neonicotinoids (e.g., Imidacloprid, Thiamethoxam): Act on the central nervous system of insects, but their use has been linked to bee population declines (Goulson, 2013).

Herbicides

Herbicides are chemical substances used to control or eliminate unwanted plants, commonly known as weeds. Weeds compete with crops for nutrients, water, sunlight, and space, reducing agricultural productivity. Herbicides help farmers and gardeners manage these unwanted plants efficiently, improving crop yield and quality (Ware and Whitacre, 2004).

Herbicides are widely used in agriculture, forestry, lawn care, and industrial settings to maintain clear land areas, prevent weed overgrowth, and reduce manual labor required for weeding (Duke, 2018).

Herbicides control weeds that compete with crops for nutrients and water. They are classified into:

Selective herbicides (e.g., 2,4-D, Atrazine): Target specific weed species without harming crops (Duke, 2018).

Non-selective herbicides (e.g., Glyphosate, Paraquat): Kill all vegetation and are used for land clearing (Benbrook, 2016).

Fungicides

Fungicides prevent or eliminate fungal diseases in crops. Major types include:

Systemic fungicides (e.g., Triazoles, Benomyl): Absorbed by plants and protect against infections from within (Hahn, 2014). Systemic fungicides (e.g., Triazoles, Benomyl): Absorbed by plants and protect against infections from within (Hahn, 2014).

Contact fungicides (e.g., Copper sulfate, Mancozeb): Remain on the surface and act as protective barriers (McGrath, 2004).

Rodenticides

Rodenticides are chemical substances specifically designed to kill rodents such as rats, mice, and other similar pests. These compounds are widely used in agricultural settings, urban pest control, and public health programs to prevent the destruction of crops, food storage, and the spread of diseases such as leptospirosis, hantavirus, and plague (Buckle and Smith, 2015).

Rodents are highly adaptable creatures capable of rapid reproduction, making effective rodent control crucial for both economic and health-related reasons. However, the use of rodenticides presents risks to non-target species, including humans, pets, and wildlife, necessitating careful regulation and application (Pimentel *et al.*,2005).

Types of rodenticides

Rodenticides are categorized based on their mode of action, toxicity level, and chemical composition.

Based on Mode of Action

1. Anticoagulant Rodenticides

Anticoagulant rodenticides are chemical compounds used for controlling rodent populations by disrupting blood clotting mechanisms, leading to fatal internal bleeding. These rodenticides are categorized into first-generation (e.g., warfarin, chlorophacinone) and second-generation (e.g., brodifacoum, difethialone) compounds. The second-generation variants are more potent and have longer biological half-lives, posing higher risks to non-target species.

Examples: First-generation anticoagulants (require multiple doses): Warfarin, Chlorophacinone.

Second-generation anticoagulants (highly toxic, lethal in a single dose): Brodifacoum, Bromadiolone (Hadler & Buckle, 1992).

2. Non-Anticoagulant Rodenticides

Non-anticoagulant rodenticides works differently from traditional coagulant poison (which prevents blood clotting). They tend to act more quickly and have different toxic mechanism. Here are the main types of non-coagulant rodenticides.

Examples: Neurotoxins: Bromethalin – disrupts energy production in nerve cells, leading to paralysis (Vandenbroucke *et al.*, 2008).

Metabolic Poisons: Zinc phosphide – releases toxic phosphine gas in the stomach, causing respiratory failure (Tobin *et al.*, 1993).

Calcium Disruptors: Cholecalciferol (Vitamin D3) – leads to hypercalcemia and kidney failure (Eason *et al.*, 2010).

NEMATICIDES

Nematicides are chemical or biological agents used to control plant-parasitic nematodes—microscopic, worm-like organisms that infest plant roots, causing severe agricultural losses. These pesticides play a crucial role in protecting crops from nematode-induced diseases, which can lead to stunted growth, wilting, and yield reduction (Chitwood, 2003).

Nematodes are a significant concern in agriculture because they attack various crops, including vegetables, fruits, cereals, and legumes. Some of the most destructive species include *Meloidogyne spp*. (root-knot nematodes), *Heterodera spp*. (cyst nematodes), and *Pratylenchus spp*. (lesion nematodes) (Jones *et al.*,2013).

Chemical Nematicides (e.g., Aldicarb, Fosthiazate): Toxic to nematodes and soil organisms (Chitwood, 2003).

Biological Nematicides (e.g., Paecilomyces lilacinus, Bacillus firmus): Use bacteria or fungi to control nematodes naturally (Nicol *et al.*, 2011).

Classification based on chemical composition

Organic pesticides

Organophosphates (e.g., Parathion, Diazinon): Highly toxic but degrade quickly in the environment (Casida & Durkin, 2013).

Organochlorines (e.g., DDT, Lindane): Persistent in the environment and banned in many countries due to bioaccumulation risks (Van den Berg, 2009).

Carbamates (e.g., Carbaryl, Methomyl): Affect the nervous system similar to organophosphates but with lower toxicity (Tomlin, 2009).

Inorganic pesticides

Derived from minerals and metals such as copper sulfate and arsenic-based compounds. These were historically used but have largely been replaced by safer alternatives (Ongley, 1996).

Biopesticides

Microbial pesticides (e.g., Bacillus thuringiensis, Trichoderma): Use bacteria, fungi, or viruses to control pests (Glare & O'Callaghan, 2000).

Botanical pesticides (e.g., Neem oil, Pyrethrin): Derived from plants and considered eco-friendly (Isman, 2006).

2.5.3 Mode of action of pesticides

Pesticides act on pests through different mechanisms, depending on their chemical nature and target sites. Here are the major modes of action of pesticides, along with citations:

1. **Neurotoxic Action**

Insecticides are principal defenses against insect pests of crops, livestock, pets, and people. Most insecticides are nerve poisons and have been since dichlorodiphenyltrichloroethane (DDT) and various polychlorocycloalkanes (PCCAs) were introduced in the 1940s, followed by organophosphates (OPs) in the 1950s, methylcarbamates (MCs) in the 1960s, pyrethroids in the 1970s, and neonicotinoids in the 1990s (Tomlin 2009.). Neurotoxicants are the major synthetic insecticides for several reasons. They act rapidly to stop crop damage and disease transmission. There are many sensitive sites at which even a small disruption may ultimately prove to be lethal. A lipoidal sheath protects the insect nerve from ionized toxicants but not from lipophilic insecticides. Poor detoxification mechanisms in nerves provide prolonged toxicant effects.

Acetylcholinesterase Inhibitors (e.g., organophosphates, carbamates): prevent the breakdown of acetylcholine, leading to continuous nerve stimulation and paralysis.

The toxicity of organophosphates and methylcarbamates to insects and mammals is attributable to inhibition of acetylcholinesterase (AChE), which is responsible for the hydrolysis of acetylcholine (ACh) at synaptic regions of cholinergic nerve endings . Acetylcholinesterase inhibitors cause acetylcholine to accumulate, resulting in excessive stimulation of cholinergic receptor. Acetylcholinesterase inhibition by Organophosphate and Methylcarbamate insecticides involves phosphorylation and carbamoylation, respectively, of serine in the esteratic site. Acetylcholinesterase is inhibited by MCs as the reversible AChE–MC complex or as the methylcarbamoylated enzyme at serine that reactivates spontaneously over a short time span. The corresponding reaction with OPs yields the phosphorylated AChE that undergoes aging or reactivates slowly, except with an oxime reactivator such as pralidoxime (Casida and Durkin, 2013).

Sodium Channel Modulators (e.g., pyrethroids, DDT): Voltage-gated Na+ channels open and close in response to changes in membrane potential (Bloomquist 1993). In the mammalian brain there are one alpha and two β subunits. The pore-forming α subunit consists of a single polypeptide chain with four internally homologous domains (I–IV), each having six transmembrane helices (S1–S6). The domain II S4-S5 linker, S5 and S6 helices, and domain III S6 helix interface the lipid bilayer and are therefore accessible to lipid-soluble ligands (Davies *et al.,* 2008). The insect Na+ channel proteins also consist of four homologous domains, each with six transmembrane segments (Soderlund. 2010).

The pyrethrins (from pyrethrum flowers), synthetic pyrethroids, and DDT, despite different origins or structures, are considered together because of similar insecticidal mechanisms. They acton axonal neurotransmission at insect voltage-gated Na+ channel recognition sites to block Na+ transport, enhance channel inactivation, prolong the course of the Na+ current during depolarization, and induce a residual slow-acting current (“tail current”) .

Pyrethroid action is considered to be of two types; Pyrethrins and synthetic pyrethroids lacking an α-cyano group, e.g., allethrin and permethrin, and the α-cyano compound fenpropathrin induce excitation (Type I action) on binding to resting or inactivated channels, shifting the voltage dependence of activation to more negative potentials and causing a slowly activating Na+ current responsible for repetitive activity. Deltamethrin and related α-cyano pyrethroids that exhibit writhing and convulsive signs in mammals (Type II action) induce profound use-dependent modification of Na+ currents, implying preferential binding to activated Na+ channel states, and recruit increasing numbers of Na+ channels into permanent open states, which results in use-dependent depolarization, inactivation of unmodified channels, and blockage of conduction. Based on these differences, consideration has been given to whether Types I and II pyrethroids should fall into the same category or be considered separately in risk analyses and residue tolerances. In all, pyrethroids and DDT keeps sodium channels open, causing hyperexcitation and paralysis (Narahashi, 2002).

GABA Receptor Antagonists (e.g., fipronil, cyclodienes): γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of insects and mammals and serves as the agonist for opening the pentameric transmembrane Cl− channel (Buckingham SD, *et al.*,2010). The human brain GABA type A receptor (GABAAR) consists of various combinations of sixteen α subunits, seven β subunits, and four γ subunits, typically two α, two β, and one γ subunit. Insect GABARs exist as several different subtypes and form a class distinct from any vertebrate GABAARs. The resistance-to-dieldrin (RDL) GABAR subtype is expressed in many insects, and RDL homomers closely mimic the pharmacology of in situ insect GABARs. Picrotoxinin (PTX), the insecticide and fish poison of the fish berry plant (*Anamirta cocculus*), was used to probe Cl− channel functions, showing that GABA opens the channel and PTX blocks Cl− flux. Several billion pounds of PCCAs, including lindane, toxaphene, and the cyclodienes such as α-endosulfan, were used in crop protection before their mode of action was established. They were joined in 1993 by the 1-phenylpyrazole fipronil, which is of lower acute toxicity to mammals. Radioligand binding and electrophysiological studies led to the recognition that the action of PCCAs and fipronil was similar to or the same as that of PTX and a series of insecticidal trioxabicyclooctanes (Casida, *et al.*, 1998). [3H]dihydroPTX was developed first as a radioligand for the PTX site, followed by the improved bicyclophosphorothionate [35S]TBPS and two bicycloorthobenzoates, [3H]TBOB for studies with mammals and particularly [3H]EBOB for investigations with insects and mammals. They all block GABAinduced signals and Cl− flux (Huang , 1997) on binding to a noncompetitive antagonist (NCA) or insecticide binding site at the receptor subunit interface. Importantly, the human GABAAR recombinant β3-homopentamer resembles the insect receptor in sensitivity and specificity for NCAs (Ratra , *et al.*,2001), prompting exhaustive site-directed mutagenesis (cysteine scanning), which pinpointed the critical active site as pore-facing residues A2` , T6` , and L9` and led to a binding site model that fits several widely diverse classes of insecticidal NCAs (Chen L, *et al.*,2006). Fipronil and cyclodienes block inhibitory neurotransmitters, leading to uncontrolled nerve activity (Bloomquist, 2003).

2. **Inhibition of Energy Production**

Mitochondrial Electron Transport Inhibitors (e.g., rotenone, pyridaben): Current insecticides and act at a relatively small range of biochemical sites, primarily located within the nervous system. A more limited number of insecticides are effective through the disruption of mitochondrial energy conservation mechanisms (Corbett *et al.*,1984). These include several newer compounds such as hydramethylnon (inhibiting electron transport at Complex 111) diafenthiuron (inhibiting F, /F,,-ATPase) Ruder *et al*.,(1992) and several types of compounds acting through mitochondrial uncoupling such as the halogenated pyrrole 4-bromo-2-(chloropheny1)- 1 -(ethoxymethyl)-5 -(trifluoromethyl)- pyrrole-3-carbonitrile (AC-303630) (Treacy *et al.,* 1994)

However, only rotenone, now a compound of limited pesticidal significance, is known to act at Complex I as an inhibitor of the respiratory chain. Complex I has recently assumed new toxicological significance, in part because of the discovery that 1 -methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP), a synthetic neurotoxin that induces Parkinsonism-like effects, may act on dopaminergic tracts through inhibition at this site.

Further clinical interest in Complex I has been generated by suggestions that deficiencies at this site may underlie several mitochondrial encephalomyopathies and neurodegenerative diseases (Hollingworth and Ahammadsahib, 1995).

2.5.4 Commonly used pesticides in cowpea storage

Phosphine Gas

Phosphine gas is widely recognized as one of the most effective fumigants for preserving stored cowpea. Its ability to infiltrate hermetically sealed storage environments makes it particularly valuable in large-scale agricultural operations. This fumigant effectively targets various stages of insect pests, including both larvae and adult forms, thereby reducing infestations significantly. Due to its gaseous nature, phosphine disperses uniformly throughout storage spaces, ensuring comprehensive pest control. However, precise application is crucial to maintaining safe concentration levels, as improper dosage or exposure can pose risks to human health and contribute to the development of resistance in pest populations (Jalali & Singh, 2003).

Despite its advantages, including its relatively minimal environmental footprint—since it naturally degrades into non-toxic compounds—phosphine misuse can lead to sublethal exposure in pests. When insect populations are exposed to doses insufficient to be lethal, they may gradually develop resistance, diminishing the gas's effectiveness over time. This growing resistance has been documented in multiple regions worldwide, raising concerns about phosphine's long-term viability in pest management strategies (Nayak *et al.*, 2013).

Dichlorvos (DDVP)

Dichlorvos, commonly abbreviated as DDVP, is an organophosphate insecticide frequently employed in the protection of stored cowpea against insect infestations. It is well known for its dual mode of action, functioning both as a contact insecticide and a vapor-phase treatment. By inhibiting acetylcholinesterase, a key enzyme responsible for nerve function, dichlorvos rapidly incapacitates and eliminates pests. Its fast-acting nature makes it particularly useful in situations requiring immediate pest control intervention. However, the continued application of dichlorvos comes with significant concerns.

One major issue with dichlorvos is the persistence of its chemical residues on stored cowpea, which can pose health risks to consumers. These residues have led to stringent regulatory scrutiny in many countries, where limits have been placed on the acceptable levels of dichlorvos in food products (Njoroge *et al.,* 2017). Prolonged exposure to this insecticide has also been associated with severe health effects, including neurotoxicity and reproductive complications. Due to these risks, the use of dichlorvos in food storage and agricultural settings remains a subject of ongoing debate and increasing regulatory restrictions.

Malathion

Another widely used organophosphate insecticide in cowpea storage is malathion. This chemical is highly effective in controlling a broad spectrum of pests, including beetles and weevils, which are common threats to stored legumes. Similar to dichlorvos, malathion works by disrupting the function of acetylcholinesterase, leading to overstimulation of the nervous system in target pests. When applied correctly, malathion can effectively reduce pest populations and prevent significant damage to stored commodities.

However, improper use of malathion, such as excessive application or failure to follow recommended guidelines, can result in residues that exceed safe limits. This raises serious health concerns for both consumers and agricultural workers who may be exposed to the insecticide. Moreover, in some regions, pests like the cowpea weevil have developed resistance to malathion, reducing its efficacy as a control measure. The emergence of resistant pest populations underscores the need for careful pesticide management, integrated pest control strategies, and alternative treatment options to mitigate the risk of resistance (Arthur, 1996).

The use of chemical insecticides and fumigants such as phosphine, dichlorvos, and malathion plays a crucial role in the protection of stored cowpeas from insect infestations. Each of these substances has distinct advantages and limitations, making it essential to apply them judiciously. While phosphine is valued for its deep penetration and minimal environmental impact, resistance development is an increasing concern. Dichlorvos, though highly effective in eliminating pests quickly, is under growing scrutiny due to health risks associated with its residues. Malathion, another effective option, is facing challenges related to overuse and pest resistance.

As pest management continues to evolve, it is imperative to explore integrated approaches that combine chemical treatments with alternative strategies, such as biological control methods and improved storage technologies. This will help sustain the effectiveness of these pesticides while minimizing the risks to human health and the environment.

2.5.5 Safety and Environmental Impacts of Pesticides

While pesticides play a crucial role in controlling pests and preventing post-harvest losses in stored cowpea, their use raises significant safety and environmental concerns. One major issue is the presence of pesticide residues in stored cowpea, which can pose health risks to consumers if the chemicals are not adequately regulated or allowed to dissipate before consumption. Certain pesticide compounds, such as organophosphates, have been linked to serious health issues, including neurotoxicity, which can affect the nervous system, leading to symptoms such as dizziness, nausea, respiratory distress, and, in severe cases, long-term cognitive impairment (Gilden *et al.*, 2010).

Additionally, exposure to pesticide residues is particularly concerning for vulnerable populations, including children, pregnant women, and individuals with pre-existing health conditions. Chronic exposure to even low levels of pesticides may contribute to endocrine disruption, immune system suppression, and increased risks of certain cancers. Inadequate storage and handling of pesticide-treated cowpea can also expose farm workers and consumers to harmful levels of these chemicals, highlighting the importance of proper pesticide management, protective measures, and adherence to established safety guidelines.

Environmental Contamination

The environmental consequences of pesticide use in cowpea storage are substantial, particularly when the chemicals are applied, handled, or disposed of improperly. During fumigation or application, pesticides may volatilize into the air or be carried away by wind, leading to atmospheric contamination and potential inhalation risks for nearby populations. Furthermore, pesticide residues can leach into the soil and groundwater, contaminating local ecosystems and endangering aquatic life, including fish and amphibians, which are highly sensitive to toxic chemical exposure. This contamination can extend to drinking water sources, posing long-term health risks to humans and animals that rely on these water supplies (Carson, 1962).

In addition to direct water pollution, pesticide runoff from storage facilities can disrupt the delicate balance of terrestrial and aquatic ecosystems. Certain pesticides, such as dichlorvos and Malathion, are known for their persistence in the environment, meaning they do not break down quickly and may accumulate over time. These persistent chemicals can have cascading effects on biodiversity, harming beneficial insects, birds, and mammals that inadvertently come into contact with contaminated food sources or habitats. For example, pollinators such as bees, which play a critical role in crop production and food security, are particularly vulnerable to pesticide exposure. Studies have shown that exposure to pesticide-treated crops or contaminated water sources can weaken bee immune systems, disrupt foraging behavior, and contribute to colony collapse, ultimately threatening agricultural productivity and biodiversity (Krupke *et al.*, 2012).

Resistance Development

One of the long-term consequences of excessive pesticide use in cowpea storage is the development of resistance among pest populations. Over-reliance on chemical pesticides, especially when used repeatedly or at sub-lethal doses, can accelerate the process of natural selection, allowing pests to develop genetic adaptations that enable them to survive future pesticide applications. Over time, resistant pest populations become increasingly difficult to control, requiring farmers to apply higher doses of pesticides or switch to more toxic and expensive chemicals to achieve the same level of pest management (Denholm & Rowland, 1992).

The emergence of pesticide-resistant pests not only increases production costs for farmers but also exacerbates environmental and health risks. As stronger chemicals are introduced to combat resistant pests, there is a higher likelihood of unintended consequences, such as the contamination of food, water, and soil with even more toxic substances. Additionally, the continued evolution of resistance among pest species can lead to a cycle of dependency on chemical interventions, reducing the effectiveness of integrated pest management (IPM) strategies that emphasize biological controls and sustainable farming practices. Addressing pesticide resistance requires a comprehensive approach that includes rotating pesticide classes, incorporating non-chemical pest control methods, and promoting research into alternative storage solutions that minimize the need for chemical interventions.

Soil and Microbial Health

Beyond their immediate impact on pests, pesticides used in cowpea storage can also have long-term effects on soil health and microbial communities. When pesticide residues enter the soil through leaching or improper disposal, they can disrupt the natural balance of beneficial microorganisms responsible for essential soil functions, such as nutrient cycling, organic matter decomposition, and nitrogen fixation. A reduction in microbial diversity can lead to decreased soil fertility, making it more difficult for crops to access the nutrients they need for healthy growth (Das *et al.*, 2014).

Soil microorganisms play a crucial role in maintaining agricultural sustainability by breaking down organic materials, enhancing soil structure, and promoting plant health. However, prolonged exposure to certain pesticides can inhibit microbial activity, reducing the soil’s ability to regenerate and support long-term crop production. Additionally, some pesticides may alter the composition of soil microbial communities, favoring harmful pathogens or opportunistic organisms that contribute to plant diseases and pest outbreaks. The loss of beneficial microbes can also impact soil aeration and water retention, making agricultural systems more vulnerable to erosion, drought, and declining productivity.

To mitigate the negative effects of pesticides on soil health, it is essential to adopt more sustainable storage practices, such as using biological pest control agents, implementing physical barriers to prevent infestations, and exploring eco-friendly alternatives to chemical fumigants. Additionally, promoting responsible pesticide application methods, such as targeted treatments and reduced dosages, can help minimize soil contamination while preserving the ecological balance needed for long-term agricultural success.

While pesticides are valuable tools for managing pests in cowpea storage, their use must be carefully managed to avoid adverse health, environmental, and agricultural consequences. The risks associated with pesticide residues in food, environmental contamination, pest resistance, and soil degradation highlight the need for sustainable pest control strategies that minimize reliance on harmful chemicals. By adopting integrated pest management approaches, improving pesticide regulation, and investing in alternative storage solutions, farmers and policymakers can work toward a safer, more sustainable agricultural system that balances productivity with environmental and human health considerations.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 RESEARCH DESIGN

For this study, cowpea (*Vigna unguiculata*) from different vendor were chosen to provide a broad analysis of pesticide residues. The cowpeas samples were categorized into eight sets, each consisting of raw samples and their corresponding milled forms. The raw samples were labeled and stored separately from the milled samples to ensure proper identification and traceability.

High-Performance Liquid Chromatography (HPLC) was used for detection. The research followed a structured process, including sample collection, preparation, extraction, purification, and analysis using HPLC.

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method was used for the initial extraction of pesticide residues because of its effectiveness in isolating pesticides from food samples (Anastassiades *et al.,* 2003). This was followed by Dispersive Liquid-Liquid Microextraction (DLLME), which improved selectivity and sensitivity before final analysis (Regueiro *et al.,* 2012). The methodology was carefully designed to ensure accuracy, consistency, and efficiency in detecting pesticide residues in cowpeas.

3.2 Sample collection and quality

Quarter bag of cowpea obtained obtained to provide a sufficient sample size for analysis. The choice of quantity ensures that multiple replicates can be analyzed for statistical reliability.

The cowpeas were sourced from local markets: Ogba market and New Benin, depending on availability and quality assessment at the time of purchase.

Three bowls were purchased to facilitate the sample preparation process, ensuring proper handling and segregation of the different cowpea varieties during the experiment.

3.2.1 Chemical and reagents

The chemicals and reagents used were of analytical or HPLC grade to ensure precision and accuracy. The following reagents were used:

Acetonitrile (1% acetic acid solution) – Primary extraction solvent.

Anhydrous magnesium sulfate ($MgSO_4$) – Removes residual water from the organic phase.

Sodium chloride (NaCl) – Enhances phase separation.

Chloroform (620 µL per sample) – Acts as an extraction solvent in DLLME.

Deionized water (4 mL per sample) – Used to induce phase separation in DLLME.

Methanol (1 mL per sample) – Used to redissolve extracted pesticide residues before HPLC analysis.

HPLC mobile phase solvents (Acetonitrile-water mixture) – Optimized for pesticide separation and detection.

Standard pesticide solutions – Used to construct calibration curves for quantitative analysis.

3.2.3 Laboratory equipments

The following equipment was used to facilitate sample preparation, extraction, and analysis:

Analytical balance – Used for accurate sample weighing.

Glass rods – Used for manual mixing.

Three bowls – Used for sample handling.

Magnetic stirrer – Used for homogeneous mixing.

Centrifuge (4000 rpm, 10 min) – Used to separate the organic phase.

Pipettes and micropipettes – Used for precise solvent measurements.

Syringes – Used for injecting the mixture into conical bottom tubes.

Syringe membrane filters (0.45µm) – Used for filtration before HPLC analysis.

HPLC system (with UV-Vis detector) – Used for pesticide residue analysis.

HPLC vials (2mL) – Used for storing filtered extracts before analysis.

3.3 Sample preparation

3.3.1 Sample collection

Cowpea samples were collected and divided into eight cowpea samples and analyzed, but the study focused on four representative samples: Sample E, F, G (containing Dichlorvos) and Sample I (containing both Dichlorvos and Cypermethrin). These four samples were chosen due to the detection of the relevant pesticides. Their results were used to represent the entire batch of eight samples, and each sample was reported in triplicates.

3.3.2 Sample preparation

A 5.0056g sample of cowpea was mixed with 10mL of 1% acetic acid in acetonitrile and stirred using a glass rod before being placed on a shaker for 30 minutes. Then, 4g of anhydrous $MgSO_4$ and 1g of NaCl were added to induce phase separation, followed by 1-minute mixing at low speed using a magnetic stirrer. The mixture was centrifuged at 4000 rpm for 10 minutes, and the supernatant was collected for further processing.

3.3.3 Pesticide Extraction

QuEChERS-Based Extraction

Weighing the sample: 5g of the homogenized cowpea sample was measured and placed in a 50mL centrifuge tube.

Solvent Addition: 10mL of 1% acetic acid in acetonitrile was added and mixed with a glass rod.

Shaking: The tube was placed in a mechanical shaker for 30 minutes.

Salt Addition: 4g of anhydrous $MgSO_4$ and 1g of NaCl were added for phase separation.

Centrifugation: The sample was centrifuged at 4000 rpm for 10 minutes.

Supernatant Collection: The organic layer (supernatant) was carefully transferred for further purification.

3.3.4 Dispersive Liquid-Liquid Microextraction (DLLME)

Supernatant Transfer: 2.5mL of the supernatant was transferred into a clean centrifuge tube.

Extraction Solvent Addition: 620µL of chloroform was added.

Water-Induced Phase Separation: The mixture was injected into a conical bottom tube containing 4mL of water.

Shaking: The tube was sealed and gently shaken for 30 seconds.

Collection of Extracted Pesticides: The chloroform layer was carefully pipetted into a clean centrifuge tube.

Solvent Evaporation: The chloroform extract was evaporated to dryness.

Reconstitution: The residue was redissolved in 1mL of methanol.

Filtration: The extract was filtered through a 0.45µm syringe membrane filter into 2mL HPLC vials for analysis.

3.4 Calculation of pesticide concentration (µg/g)

The pesticide concentration was calculated using the following formula:

$y=ax+b$

y is the pesticide concentration (µg/mL),

a is the slope (from calibration curve),

x is the measured peak area from HPLC,

b is the intercept(from calibration curve).

3.5 Data collection methods

Data collection involved recording chromatographic retention times, peak areas, and concentrations for each pesticide in Sample A and Sample I. These values were documented and multiplied across all eight samples to represent triplicate results.

3.6 Data analysis

The data obtained from HPLC analysis were processed using chromatographic software. The retention times and peak areas of the detected pesticide residues were compared with standard reference values. Quantitative analysis was performed by establishing calibration curves for the identified pesticide compounds. Statistical analysis was conducted to assess variations among different samples. The results were interpreted in relation to permissible pesticide residue limits to determine contamination levels and potential health risks.

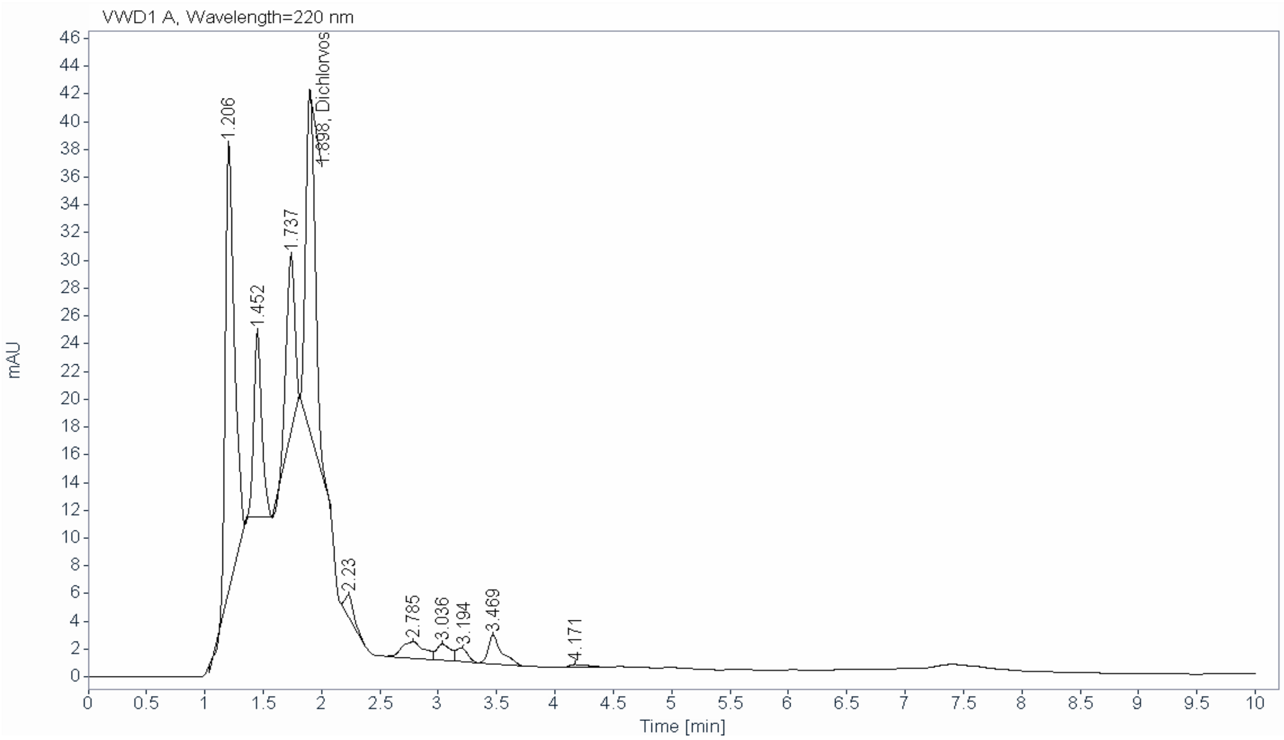
CHAPTER FOUR

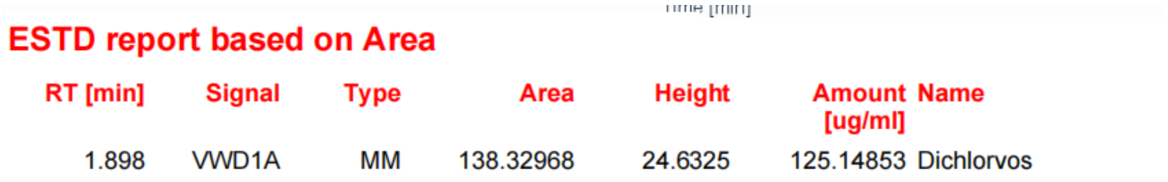
RESULTS

4.1 CHROMATOGRAM OF STANDARD PESTICIDE SOLUTION

The standard pesticide solution was generated using a chromatogram to establish reference retention times for each pesticide. The calibration curve was used to determine the concentration of pesticides in the cowpea samples.

Sample E

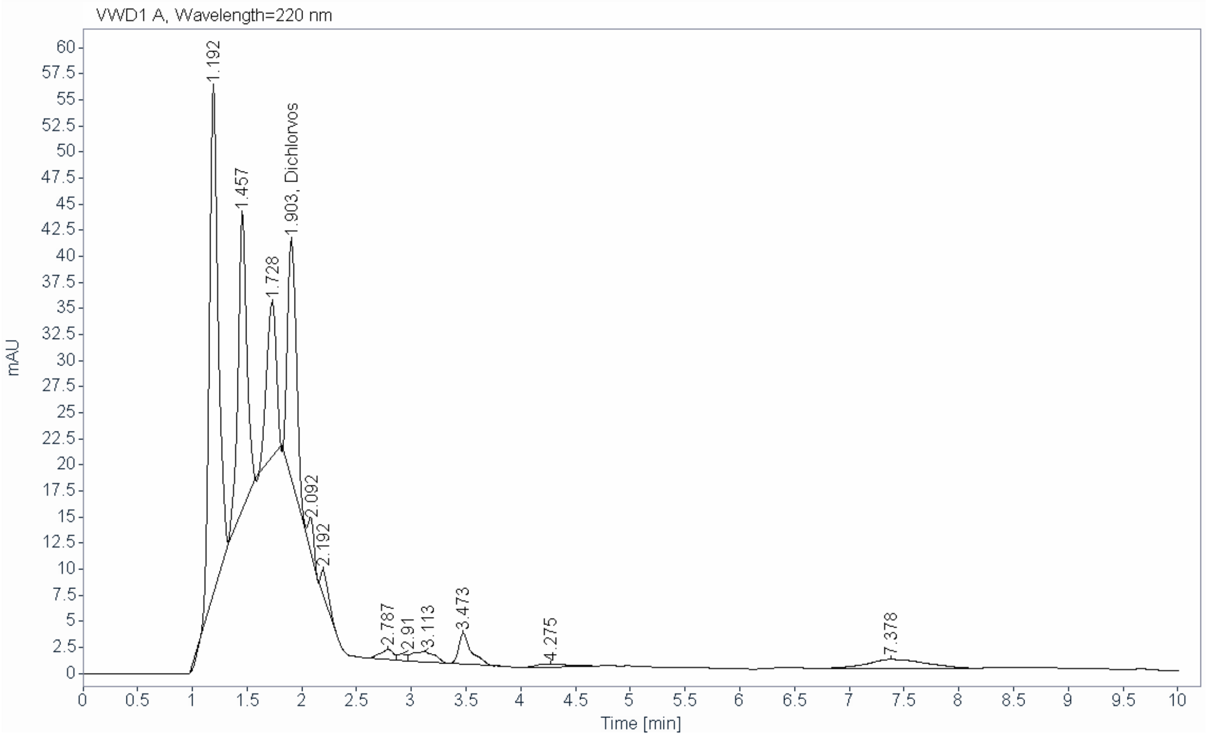




ESTD report based on Area

RT [min]	Signal	Type	Area	Height	Amount [ug/ml]	Name
1.898	VWD1A	MM	138.32968	24.6325	125.14853	Dichlorvos

SAMPLE F

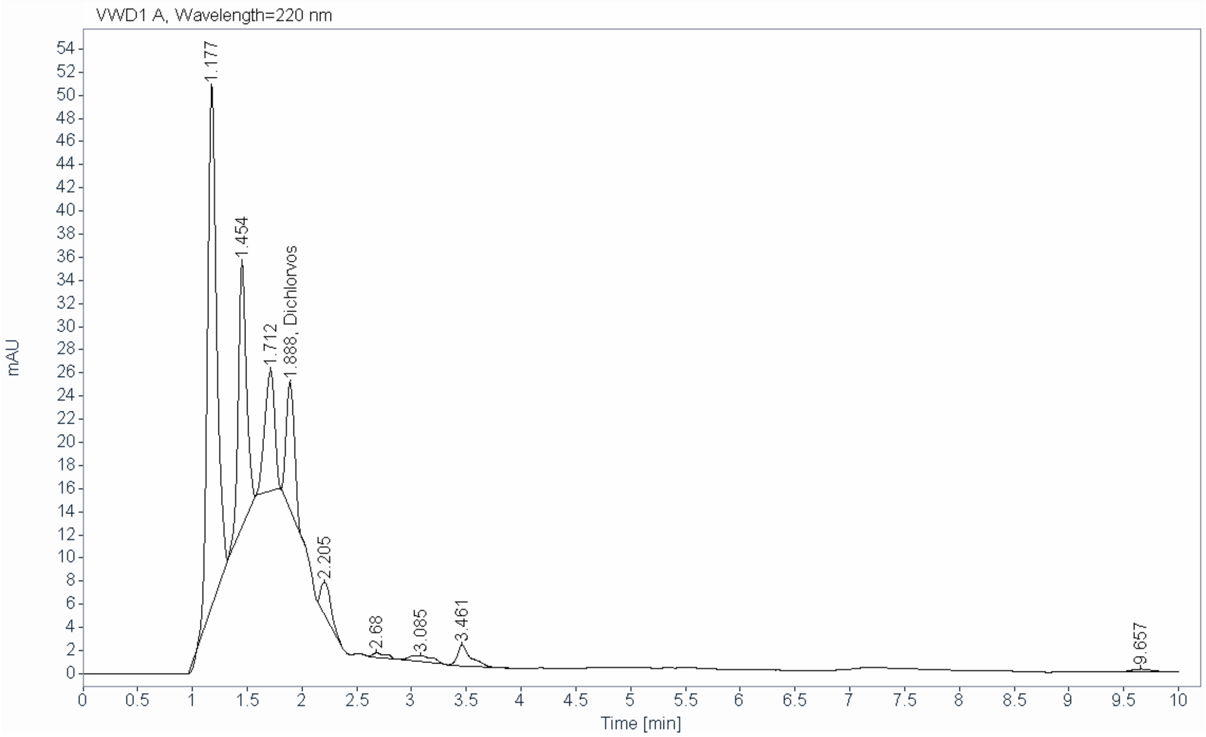


Time [min]

ESTD report based on Area

RT [min]	Signal	Type	Area	Height	Amount [ug/ml]	Name
1.903	VWD1A	MM	128.28348	22.7357	116.05961	Dichlorvos

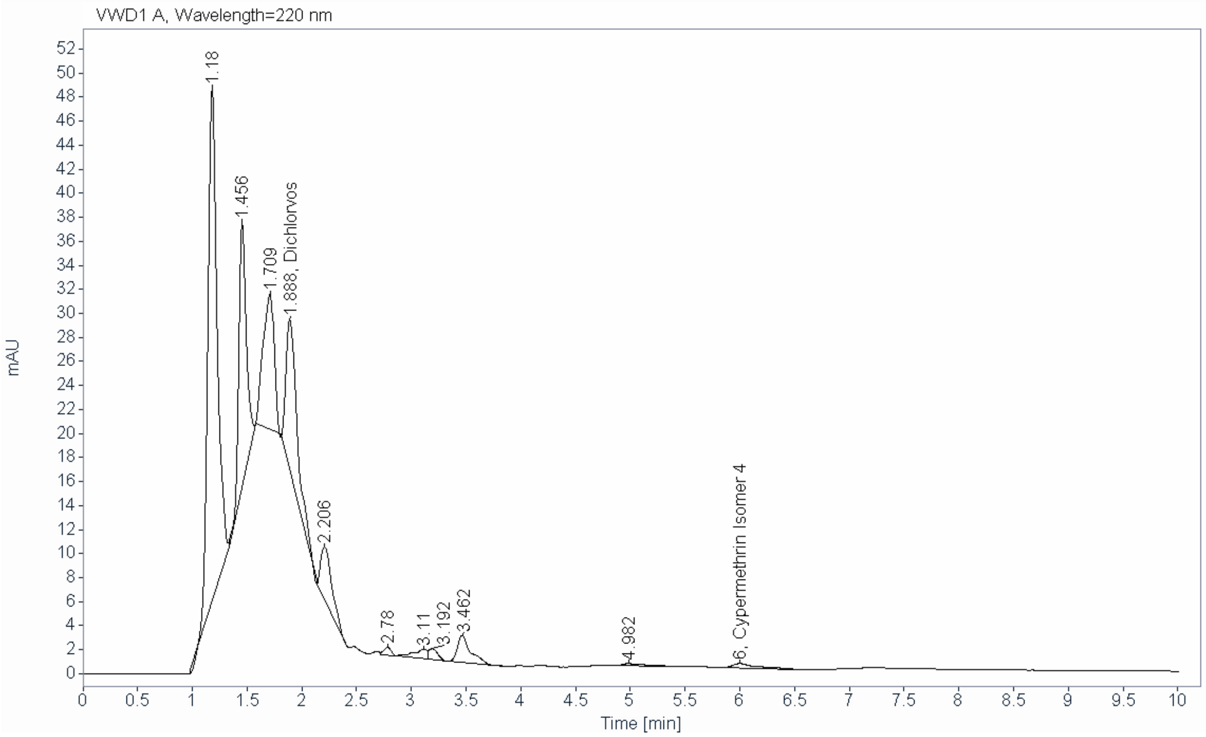
SAMPLE G



ESTD report based on Area

RT [min]	Signal	Type	Area	Height	Amount [ug/ml]	Name
1.888	VWD1A	MM	58.28262	10.8632	52.72898	Dichlorvos

SAMPLE I



ESTD report based on Area

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

4.2 PESTICIDE RESIDUE CONCENTRATION IN COWPEA SAMPLES

The concentrations of Dichlorvos and Cypermethrin in the analyzed samples were calculated and expressed in µg/ml to indicate residue levels in the cowpeas.

Table 4.1: Pesticide Residue (Dichlorvos) Concentration in Cowpea Samples (µg/mL)

Samples	Retention time (min)	Peak area	Concentration (µg/mL)

E	1.898	138.33	125.15
F	1.903	128.28	116.06
G	1.888	58.28	52.73
I	1.888	87.90	79.52

Table 4.2: Pesticide Residue (Cypermethrin Isomer 4) Concentration in Cowpea Samples (µg/mL)

Samples	**Retention time (min)**	**Peak area**	**Concentration (µg/mL)**
I	6.000	5.33	2.82

4.3 STATISTICAL ANALYSIS

To evaluate the data consistency and precision, statistical analysis was performed calculating key metrics including mean concentration, standard deviation and relative standard deviation (RSD).

Mean concentration $\overline{x} = \frac{\sum x_i}{n}$ = 93.365 µg/ml

Standard Deviation (SD)= $\sqrt{\frac{\sum(x_i - \overline{x})^2}{n-1}}$ =33.86 µg/ml

Relative Standard Deviation (RSD) = $\frac{S.D}{\overline{x}} X\ 100$

$= \frac{33.86\mu\frac{g}{ml}}{93.365\mu\frac{g}{ml}}\ x\ 100$

= 36.27%

Where

x_i= each individual concentration

$\overline{x}$ = mean concentration

n = total number of sample

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussions

This research evaluated the quantitative analysis of pesticide residues (Dichlorvos and Cypermethrin) in cowpea samples using chromatographic techniques (The retention times and peak areas were used to determine the concentration of pesticide residues, which were expressed in µg/mL.

The concentrations of Dichlorvos in the analyzed cowpea samples varied: Sample E had the highest concentration (125.15 µg/mL), followed by sample F (116.06 µg/mL). Sample I had a moderate concentration (79.52 µg/mL). Sample G had the lowest concentration (52.73 µg/mL). These findings indicate that pesticide residue levels differ among samples, potentially due to varying pesticide application methods, time intervals between pesticide application and sample collection, or environmental degradation.

Cypermethrin was only detected in sample I at a concentration of 2.82 µg/mL. The retention time (6.000 min) and peak area (5.33) confirm the presence of Cypermethrin but at a significantly lower concentration compared to Dichlorvos. The lower concentration of Cypermethrin could be attributed to its lower application rate, higher degradation rate, or different chemical properties that affect its persistence in cowpeas.

Statistically,

The mean concentration of Dichlorvos across all samples was 93.365 µg/ml, The standard deviation (SD) was 33.86 µg/mL, indicating a moderate spread of data around the mean. The relative standard deviation (RSD) was 36.27%, reflecting variability among the samples. A high RSD suggests significant variations in pesticide residue levels among different cowpea samples, possibly due to inconsistent pesticide application or varying degradation rates.

Pesticide residue levels in food products are regulated by international and national agencies such as the Codex Alimentarius Commission, the U.S. Environmental Protection Agency (EPA), and the European Food Safety Authority (EFSA). The Maximum Residue Limit (MRL) for Dichlorvos and Cypermethrin in cowpeas varies across different regulations. The European Union (EU) has set an MRL of 0.01 mg/kg for Dichlorvos in cowpeas (EFSA, 2021). The U.S. EPA allows up to 0.1 mg/kg of Dichlorvos in raw agricultural products (EPA, 2022). The MRL for Cypermethrin in cowpeas is 0.05 mg/kg under Codex Alimentarius standards (FAO/WHO, 2020).

The detected Dichlorvos concentrations (52.73 – 125.15 µg/mL) in this study significantly exceed the maximum residue limits (MRLs) set by international food safety authorities. Similarly, Odebunmi *et al.,* (2019) found Dichlorvos levels ranging from 60 to 130 µg/mL in legumes from Nigerian markets, with most samples surpassing WHO's recommended MRL of 0.01 mg/kg. Another study by Mekonen *et al.,* (2021) detected Dichlorvos concentrations up to 105 µg/mL in Ethiopian cowpeas, suggesting that excessive pesticide application before harvest is a common practice in developing countries.

Furthermore, research by González-Rodríguez *et al.*, (2011) found that Dichlorvos residues in vegetables and grains degrade slowly, particularly in warm and humid climates, where volatilization and photodegradation are less effective. This aligns with this study, as the high residue levels in the cowpeas could be attributed to inadequate degradation due to environmental factors and non-adherence to pre-harvest intervals. Cypermethrin was detected in only one sample (2.82 µg/mL), which is significantly below the Codex Alimentarius MRL of 0.05 mg/kg (FAO/WHO, 2021). This is consistent with studies by Tudi *et al.,* (2021) and Akan *et al.,* (2019), who reported that Cypermethrin residues in grains tend to be lower than organophosphates due to their higher photodegradation rate and lower systemic absorption. Similarly, Adeyemi *et al.,* (2022) found that Cypermethrin levels in cowpeas from West Africa were non-detectable in 70% of samples, suggesting that its rapid degradation reduces its persistence in stored food products.

The low Cypermethrin levels in this study also align with the findings of Iqbal *et al.*, (2020), who analyzed pesticide residues in legumes from South Asia and observed that Cypermethrin concentrations rarely exceeded 0.01 mg/kg due to its low soil and crop retention capacity.

The relative standard deviation (RSD) of 36.27% indicates high variability in pesticide residue levels among the samples. This observation is consistent with Mansour *et al.,* (2020), who found that pesticide residue levels vary widely due to differences in pesticide application practices, environmental conditions, and post-harvest storage methods. Similarly, Mekonen *et al.,* (2021) reported RSD values above 30% in pesticide residue analysis, which they attributed to inconsistent pesticide application and differences in crop absorption rates.

These findings emphasize the need for stricter regulation and monitoring of pesticide use in agricultural production.

Pesticide residues in food pose serious health risks, especially when they exceed safe limits. The toxicological risks of pesticide residues are well documented. High Dichlorvos exposure has been associated with neurological disorders, reproductive toxicity, and potential carcinogenicity (WHO, 2021). Research by Badii and Landeros (2020) showed that chronic exposure to organophosphate pesticides, particularly Dichlorvos, can disrupt acetylcholinesterase activity, leading to neurotoxic effects. This supports the concern that exceeding MRLs in food products poses serious health risks.

In contrast, Cypermethrin has lower acute toxicity, but studies have linked high exposure to skin irritation, endocrine disruption, and neurotoxic effects (Gilden *et al.,* 2018). The low Cypermethrin concentration detected in this study suggests a reduced risk, but its cumulative exposure from multiple food sources could still be a concern.

Alternative Pest Control Methods which include Biopesticides and integrated pest management (IPM), Neem extracts, Bacillus thuringiensis (Bt), and pheromone traps are effective eco-friendly alternatives which can be employed in the agricultural sector to reduce reliance on chemical pesticides (Isman, 2006).

Also, Routine residue monitoring in food markets can prevent contaminated products from reaching consumers (FAO/WHO, 2021). Public education on pesticide risks and proper food washing methods can help reduce exposure (Gilden *et al.*, 2018).

5.2 Conclusion

The study revealed high Dichlorvos concentrations in cowpea samples, exceeding permissible limits, while Cypermethrin levels were minimal. These findings highlight potential health risks and the need for stricter pesticide regulation and better agricultural practices. Implementing sustainable pest control measures and ensuring compliance with food safety standards can help mitigate pesticide contamination in food.

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