EVALUATION OF THE EFFECT OF SODIUM BICARBONATE (BAKING SODA) ON THE REMOVAL OF ORGANOCHLORINE PESTICIDES; LINDANE AND ENDOSULFAN FROM COWPEAS (*Vigna unguiculata*)

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JANUARY, 2023.

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARMD) DEGREE.

JANUARY, 2023.

CERTIFICATION

This is to certify that this project was carried out by IFEOMA ROSELINE NWAFOR of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

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SUPERVISOR DATE

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DR E. E. ODION

HEAD OF DEPARTMENT DATE

DEDICATION

This study is dedicated to my dear mother, Mrs. Patience M. J. Nwafor for being the best and most supportive mum ever!

ACKNOWLEDGMENTS

Firstly, I sincerely thank the one who makes everything beautiful in His own time; the one and only Almighty God for his grace, mercies and favour upon my life all through my sojourn in the University of Benin.

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ABSTRACT

Organochlorine pesticides, such as lindane and endosulfan are widely used in Agriculture. They have been reported to have negative impacts on human health and the environment. The decontamination of food crops from these pesticides is therefore of great importance. In this study, the effectiveness of 10% Sodium bicarbonate in removing the aforementioned pesticides from cowpeas was evaluated. Sodium bicarbonate is a natural and inexpensive substance that has been shown to be effective in removing other types of pesticides from food crops. The results of this study showed that treatment with sodium bicarbonate was able to significantly reduce the levels of lindane and endosulfan on the cowpeas. This suggests that sodium bicarbonate is a promising option for the decontamination of cowpeas and potentially other food crops from organochlorine pesticides. Further research is needed to fully understand the optimal conditions and mechanisms for using sodium bicarbonate as a decontaminating agent and to determine its potential for use in other crops and in different regions.

CHAPTER ONE

1.0 INTRODUCTION / LITERATURE REVIEW

1.1 BACKGROUND

Pesticides are extensively utilized in agriculture to kill undesirable organisms that could harm crops. As a result, pesticides improve agricultural yield, prevent insect infestations, and increase farm productivity. Pesticides are a broad category of chemicals that include insecticides, rodenticides, herbicides, biocides, etc. Most of these pesticides act by disrupting the physiologic activities of the target organism thereby resulting in a dysfunction and reduced vitality of target organism.

1.2. HISTORY OF PESTICIDE USE

Pesticides have been used to protect crops since ancient times, possibly even before 2500 B.C.E. In Sumeria, around 4,500 years ago, elemental sulfur was utilized as the first pesticide. By the fifteenth century, crops were being treated with poisonous substances like arsenic, mercury, and lead to kill pests (Miller, 2002).

Tobacco leaf extract known as nicotine sulfate was employed as an insecticide in the 17th century. Two other organic pesticides were introduced in the 19th century: rotenone, which is derived from the roots of tropical crops, and pyrethrum, which comes from chrysanthemums (Miller, 2002).

Dichlorodiphenyltrichloroethane (DDT), which is a highly efficient insecticide, was developed by Paul Müller in 1939. It swiftly rose to prominence spot among pesticides used globally. Later research revealed that DDT posed a danger to biodiversity because it prevented many fish-eating birds from breeding (Ritter, 2009).

DDT is still utilized in some underdeveloped countries to prevent malaria and other tropical diseases by eliminating mosquitoes and other disease-carrying insects, even though it is currently prohibited in at least 86 countries. (Elizabeth Miller, 2012.).

1.3 CLASSIFICATION OF PESTICIDES

Pesticides may be classified based on the following:

1. Chemical nature (organochlorines, organophosphates, carbamates, Pyrethroids and Phenyl amides).
2. Application requirement (agriculture, public health and domestic).
3. Target organism or targeted use (insecticide, herbicide and fungicide).

Table 1.0: Classification of pesticides based on their chemical nature.

No	Chemical Group	Selected Examples
1	Organochlorines	Lindane, Chlordane, Heptaclor, Endosulfan Dichlorodiphenyltrichloroethane, Dicofol, Eldrin, Dieldrin.
2	Organophosphates	Dimefox, Mipafox, Methyl Parathion, Fenthion, caumphos, Dichlorovos, Malathion
3	Carbamates	Prupoxur, Carbofuran, Aminocarb, Aldicarb, Diallate, Butylate, Cycloate, Thiourea.
4	Pyrethroids	Allethrin, Dimethrin, Tetramethrin, Cyclethrin, Cypermethrin
5	Phenyl amides	Carbanilates

(Jayaraj *et al*., 2016)

1.4 REGULATION OF PESTICIDE USE

Before being marketed or used, pesticides often need to be licensed by a government body. For instance, the National Agency for Food and Drug Administration and Control, NAFDAC, is the agency that oversees pesticide usage in Nigeria. The effectiveness and safety of the insecticides must be determined through extensive and rigorous studies. A label that specifies how to utilize the material properly is made during the registration process.

Pesticides are given a toxicity Class based on their acute toxicity. Certain pesticides are labeled limited use pesticides because they are deemed too dangerous to be sold to the general public.

Restricted use pesticides may only be purchased or administered by qualified applicators who have passed an exam. Governmental organizations tasked with enforcing pesticide laws may audit sales and use records, which must be kept on record (USEPA, 1990).

1.5 NEGATIVE EFFECTS OF PESTICIDE USE

Pesticides have wide-ranging consequences that are evident in many facets of our environment, from the ecosystem itself to the farmers or individuals who use them to even the consumers.

Acute or chronic ailments like abdominal pain, headaches, nausea, vomiting, skin and eye problems, among others, have been linked to exposure to particular pesticides in farmers. The main way that consumers are exposed to pesticides is through their diet, which could result in serious health threats (Damalas and Koutroubas, 2016)**.**

Pesticide use over the years has become a public health concern owing to the health and environmental risks associated with the inappropriate exposure of some types of pesticides. A study suggests that only a small percentage (0.3%) of applied pesticides goes into the target pest while 99.7% go somewhere else into the environment (Pimentel, 1995)

According to a World Health Organization study, developing nations use 80 percent of all pesticides (Veil, 1990). Agricultural workers from developing countries have been exposed to excessive quantities of agricultural chemicals, including pesticides, as a result of improper market regulations, inadequate legislation, and total ignorance. (Smith and Jong, 2001).

This has resulted in the restriction and regulation of their industrial and agricultural uses. Of particular concern are the organochlorines (also called chlorinated hydrocarbons), which have been extensively used worldwide because of their prolonged period of action, low cost and toxicity against various pests. They represent one of the first categories of pesticides ever synthesized and are used in agriculture. Most of them are usually used as insecticides for the control of a broad range of insects, and have long-term residual effect in the environment. Common examples of these pesticides include: dichlorodiphenyltrichloroethane (DDT), lindane, endosulfan, aldrin, dieldrin, heptachlor, toxaphene, chlordane, etc. Organochlorine pesticides are characterized by their bioaccumulation in the environment, especially in the food chain, where they find their way into the human body and pose serious health risk to consumers (Raslan *et al*., 2018).

1.6 ORGANOCHLORINE PESTICIDES- CHEMISTRY, PERSISTENCE AND BIOCHEMICAL TOXICITY.

The organochlorine pesticides are organic compounds attached to five or more than five chlorine atoms. The basic characteristics of organochlorine pesticides include high persistence, low polarity, low aqueous solubility and high lipid solubility. These pesticides enter the environment following application, polluted wastes discarded into landfills, and industrial wastes discharged into the environment (Jayaraj *et al*., 2017).

Organochlorine toxicity is mainly due to stimulation of the central nervous system. they have affinity for the α-subunit of the voltage-dependent sodium channels in neurons; they prevent their closing and this results in repetitive firing of action potentials and the release of neurotransmitters (Karami-Mohajeri and Abdollahi, 2011). Primary adverse neurological effects of Organochlorines also result from inhibition of $GABA_A$ and glycine receptors (Heusinkveld and Westerink, 2012).

According to reports, there is a link between using organochlorine pesticides and an increased risk of cancer, as well as neurological and reproductive disorders (Prabhu *et al*.,2009).

Organochlorines interfere with the endocrine system's molecular circuitry and function, acting as endocrine disrupting chemicals (EDCs). (Sohail *et al*., 2004). According to the (U.S.) Environmental Protection Agency, endocrine disrupting chemical (EDC) is defined as “a chemical that interferes with the function of the endocrine system by mimicking a hormone, blocking the effects of a hormone, or by stimulating or inhibiting the production or transport of hormones”. Chemicals that act as EDCs may alter the levels of endogenous hormones particularly the steroid hormones such as estrogen and also thyroid hormones (Lee *et al*., 2013).

Pesticide exposure causes neuromuscular problems and stimulates the metabolism of drugs and steroid hormones whether it is direct or indirect. (Subramaniam and Solomon, 2006). Another mode of exposure to these pesticides is through diet. Among food items, fatty food such as meat, fish, poultry, and dairy products serve as main causes (Rusiecki *et al*., 2008). Many of the organochlorine molecules are carcinogens and neurotoxic (Kaiser, 2000).

The menace caused by endosulfan is of great concern. Endosulfan lingers in the environment for prolonged periods of time and bioaccumulates in plants and animals, contaminating human food. (Briz *et al*., 2011). It affects mainly the central nervous system and was found to have higher acute inhalation toxicity than dermal toxicity. Gastrointestinal absorption of Endosulfan is very high (USEPA, 2010). Some of these pesticides were found to increase levels of free thyroxine (T4) and total triiodothyronine (T3) and to have an opposite relationship with thyroid-stimulating hormone (TSH), which can result in an imbalance of thyroid hormones and abnormal thyroid hormone level. (Meeker *et al*., 2007).

Organochlorine pesticides were reported to increase the risk of hormone related cancers including breast, prostate, stomach and lung cancer (Wolff *et al.*, 1993).

Organochlorine compounds are highly lipophilic and thus can accumulate in fat-rich food such as meat and milk (Hernandez *et al*., 1994).

The most common type of pesticide used by farmers in Nigeria is lindane (Gammalin 20®), which is used in aqueous solutions to prevent a variety of insect pests as well as to formulating other kinds of unauthorized pesticides that are administered locally to crops. (Ogunfowokan *et al*, 2012).

1.7 LINDANE

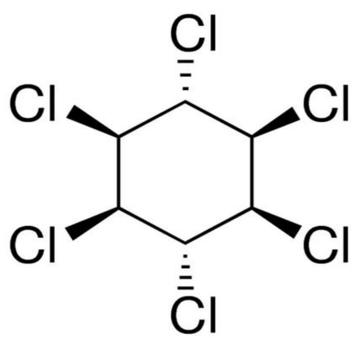


fig 1.0 Structure of lindane

Chemical name: 1,2,3,4,5,6-hexachlorocyclohexane.

Other names: *gamma*-hexachlorocyclohexane (γ-HCH), gammaxene, Gammallin

And gamma: benzene hexachloride (γ-BHC)

Molecular formula: C6H6Cl6

Molecular weight: 290.81g/mol

Description: whitish crystalline powder

Odour: musty

Solubility: poorly soluble in water and volatile in air, readily soluble in organic solvents such as Ethyl alcohol, Ethyl ether, Benzene, Dichloromethane, Chloroform.

It is an organochlorine chemical and a gamma isomer of hexachlorocyclohexane that has been used both as an agricultural insecticide and as a pharmaceutical agent in the treatment of lice and scabies infestations.

There are numerous lindane isomers, but only six of them including the α-, β-, γ-, δ-, ε- isomers are relatively stable. Though only the gamma isomer has insecticidal properties (Brooks, 1977). The International Organization for Standardization (ISO) common name and the WHO specification use "Lindane" to refer to material containing >99% γ-HCH.

Lindane was originally synthesized in 1825 by Michael Faraday. It is named after the Dutch chemist Teunis van der Linden (1884–1965), the first to isolate and describe γ-hexachlorocyclohexane in 1912.

In 2009, the production and agricultural use of lindane was banned under the Stockholm convention on persistent organochlorine pollutants (Vijgen *et al.,* 2011).

1.7.1 Lindane toxicity

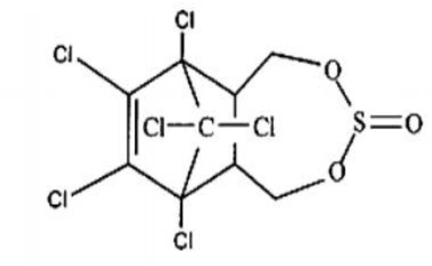
Humans are exposed to lindane through ingestion, inhalation, or direct skin contact. Numerous harmful impacts on human and animal health have been linked to it. Chronic exposure to high levels of lindane in humans has negative effects on the nervous system, liver, and kidneys and may be carcinogenic since the majority of lindane evaluations have found that it may probably cause cancer. (Loomis *et al,* 2015).

However, most cases of its usage occur in agriculture for seed and soil treatment. Its use has been banned in various countries including the European Union countries due to its highly toxic nature. Symptoms of Lindane toxicity include convulsions, confusions, nausea, vomiting, respiratory depression, unconsciousness (Goel and Aggarwal, 2007).

1.7.2 Mechanism of toxicity:

Lindane toxicity is mainly due to stimulation of the Central Nervous System, it inhibits the Gamma Amino Butyric Acid (GABA) mediated transmission by interfering with GABA-A receptor chloride channel complex at the picrotoxin binding site.

1.8 ENDOSULFAN



Endosulfan

Fig. 2.0 Structure of Endosulfan

Chemical name: 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide.

Other names- Thiodan, Thiodon, Thiotox, Benzoepin.

Chemical formula- C9H6Cl6O3S

Molecular mass- 406.90g/mol

Appearance- Brown crystals

Solubility- Denser than water and nearly insoluble in water, soluble in organic solvents.

Odour- pungent, slight sulfur dioxide odour.

Endosulfan is an organochlorine insecticide that belongs to the cyclodiene group. It has 2 isomers; α- and β-. The technical grade Endosulfan is a mixture of two stereoisomers- α- and β-Endosulfan, in a ratio of 7:3 (Verma *et al*., 2011). It is extensively applied in agriculture to protect crops (Li *et al*., 2009).

Due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor, it is being phased out of use on a global scale.

Following its dangers to human health and the environment, its manufacture and usage were negotiated for a prohibition in the Stockholm Convention in April 2011.

1.8.1 Endosulfan toxicity

Humans are exposed to Endosulfan through contaminated food or skin contact.

The human body experiences physiochemical changes as a result of Endosulfan.

It is found in fatty tissue, human milk, placenta, and umbilical cord in women, with higher concentrations found in adipose tissues than in either human milk or placenta. (Cerrillo *et al.,* 2005). Endosulfan is an endocrine disrupting compound which can enter into the body through different modes. It could change the mechanism of MAPkinase signaling which lead to changes in survival, growth and proliferation of epithelial cell resulting in cancer (Ledirac *et al*., 2005). Longer exposure to it sometimes gives rise to permanent neurological disorders which directly affects the CNS.

Symptoms of Endosulfan toxicity include- confusion, headache, agitation, diarrhea, vomiting, nausea, oedema, etc.

1.9 APPLICATION OF PESTICIDES IN THE CULTIVATION OF THE COWPEA.

Cowpea (*Vigna unguiculata*) is a leguminous crop of the tribe Phaseolae, family Leguminosae, widely grown in tropics and subtropics for human as well as for animal consumption (Bliss, 1972).

Worldwide, dried cowpea production exceeds 5.4 million tons, with Africa accounting for almost 5.2 million. Nigeria is the world's greatest consumer and producer, accounting for 58% of global production and 61% of production in Africa. (FAOSTAT, 2012). Cowpeas cultivated in Nigeria differ in their seed size, seed coat texture and colour. Brown cowpeas (beans) and white cowpeas (beans) are common. The plant is mostly produced for its seeds, which are known as beans in the region. Beans can be eaten raw when they are semi-ripe, as a pulse when they are dry and mature, or crushed into flour.

Cowpeas are a fantastic source of vitamins, minerals, proteins, and calories.

Cowpea seeds are low in anti-nutritional factors and can contain up to 25% protein. (Rangel *et al*., 2003).

This diet complements the mainly cereal diet in countries that grow cowpeas as a major food crop. Despite the importance of the cowpea crop for human welfare and needs, it is faced with the problems of pest infestation right from the field to storage. Jackal and Daoust (1986) reported that insects are a major factor in the low yields of African cowpea crops, and they affect each tissue component and developmental stage of the plant. In heavy infestations, insect pressure is responsible for over 90% loss in yield.

Prevention clearly surpasses post-harvest control methods in food intended for human consumption. Hence the need for an efficient pre-harvest management which must render the grain inhospitable to insects. (Keri, 2009). This is usually achieved to some extent by either the use of organic pesticides which are not as effective as synthetic pesticide or planting insect resistance varieties.

In order to prevent the attack of these crops by pests both on the field and in storage, pesticides are applied on them, and often times, the pesticides were applied indiscriminately by the farmers (Lowenberg-DeBoer and Ibro, 2008).

Farmers and retailers that have little to no knowledge on how to apply pesticides or their effects on health when they are present in food frequently misuse, overuse, or use them inappropriately. As a result, foodstuffs to which pesticides are applied retain dangerous levels of pesticide residues or metabolites that have been adsorbed.

Ingestion of contaminated foods, inhalation, and cutaneous exposure are the most frequent methods of exposure to pesticide residues, and each of these methods carries a potentially substantial risk to one's health. Several cases of food poisoning after consumption of meals prepared from cowpea grains and bean that were suspected to contain appreciable amount of organochlorine insecticide residues have been reported (Adedoyin *et al*., 2008; Adeleke, 2009). For instance, in northern Nigeria, about 95% of stored cowpea and bean are treated with insecticides, and farmers use these pesticides even when they are not required (Lowenberg-DeBoer and Ibro, 2008).

Food and Agriculture Organization/World Health Organization (FAO/WHO; 1990) stated all over the world about 3 million people are poisoned and 220,000 die each year from insecticide poisoning, most of which occur in the developing countries, even though far greater quantities are used in the developed countries (Bhanti *et al.*, 2004). In Nigeria, for instance, Shaibu (2008) reported that two children and 112 people were hospitalized after eating cowpea treated with pesticides in Cross Rivers state; 20 fast-food outlets were shut down in Nigeria as a result of deaths linked to pesticide residue in their products (Chikwe, 2010); and 116 students of a school in Doma, Gombe State, fell ill and were hospitalized after eating a meal contaminated by pesticide (Adegbola *et al*., 2012).

A study was conducted by Gwary *et al*., (2011) to measure residues of pesticides in samples of the common bean (*Phaseolus vulgaris*); a similar crop as the cowpeas, collected in six local government areas of Borno state, Northeast Nigeria. This study revealed that DDT, Dichlorovos and Eldrin are heavily utilized in storage of beans from insect attack and suggested that strict monitoring of pesticide use in agriculture and food storage is required.

Several studies have been and are currently being conducted to determine the best ways to remove pesticide residues from conventional food products such as vegetables and legumes treated with high-risk pesticides.

In one of such studies Odion *et al*., (2021) assessed the impact of common household processing techniques such soaking, washing in hot water and dehulling cowpeas on the pesticide residues of lindane and Endosulfan. The study findings revealed that dehulled cowpeas contained no pesticides or pesticide residues which may indicate that these pesticides do not permeate the testa of the cowpeas.

However, traditional methods of preparing cowpeas for consumption most times do not require dehulling, except in special delicacies as in the Moin-Moin (Bean cake) snack or the popular African *Akara*. This study seeks to evaluate the effect of 10% sodium bicarbonate solution on cowpeas impregnated with varying concentrations of Lindane and Endosulfan respectively without dehulling.

1.10. JUSTIFICATION OF STUDY

The Cowpea (*Vigna unguiculata*) is a commonly cultivated crop in Nigeria. It is a favourite to a good number of the Nigerian populace and this is attributed to the variety of delicacies that can be prepared from it. Besides its various delicacies, it is highly nutritious and provides the body with numerous health benefits; it is a rich source of protein, carbohydrate, vitamins etc. thereby making it an indispensable meal in several homes today.

At every stage of development, a wide variety of insects severely attack the cowpea. In storage, the crop is attacked by weevils (*Callosobruchus maculatus* Fab) which is regarded as the major insect pest on the crop (Singh *et al*., 1990). Because of this, farmers are left with no choice but to utilize tolerant varieties and insecticide sprays. (Dugie *et al*., 2009), some of which pose serious health threats to human and animal health.

According to reports, Nigerian agricultural products were rejected at international export markets because they contained significant pesticide residues (New Telegraph 2017).

In 2015 and 2016, the European Union (EU) rejected 67 processed and semi- processed Nigerian products, including white (cowpeas) and brown beans. The ban is yet to be lifted due to failure to comply with international food standards.

In addition, it has been reported that levels of pesticide residues brought on by inappropriate application and repeated sprays have been linked to poisoning and fatalities of individuals across both rural and urban areas of Borno state and in Nigeria.

An incidence of food toxicity in Lagos and Gombe states, Nigeria had led to death and hospitalization of many people (Mada *et al*, 2014). Thus, it is important to

investigate the pesticide residues present in grains that are in storage and how they are being affected by conventional household processing.

In addition to the improved agricultural yield following the use of Pesticides, particularly the organochlorines in the cultivation of cowpea, man is exposed to several health risks and the ecosystem at large is threatened by the bioaccumulation of these pesticides. Hence there is a need to evaluate possible methods of decontaminating the cowpea from these unhealthy pesticides, ultimately rendering it more suitable for consumption and subsequently enlighten the public on these methods of decontamination.

1.11. OBJECTIVES OF STUDY

The objectives of the study include;

1. To evaluate the effect of 10% Sodium bicarbonate on the removal of organochlorine pesticides; Lindane and Endosulfan from cowpeas (*Vigna unguiculata).*
2. To quantitatively determine the extent of decontamination by comparing the pesticide content before and after the decontamination process using GC-MS analysis.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Apparatus

Hot-air oven (Jenlab)

Electronic blender

Electronic weighing balance (G & G)

Soxhlet apparatus

Heating mantle (Bosch)

Water bath (Stuart)

Temperature regulator

Refrigerator

Stop watch

Digital thermometer

Measuring cylinders (50 mL, 25 mL)

Conical flasks (250 mL)

Round-bottom flasks (250 mL)

Beakers (100 mL, 250 mL)

Separating funnel

Aluminum foil

Retort stand

Spatula

Glass stirrer

Glass funnel

Thimble

Plastic sieve

Cotton wool

Micropipette

Anti-bumping agents

Sample tubes.

2.2 Reagents and Chemicals

Distilled water

Dichloromethane (DCM) supplied by Labscan (Sigma- Aldrich, India)

Silica gel from Oxford laboratory (India)

Anhydrous sodium sulphate

Sodium bicarbonate (Amel Susan®).

Reference standard pesticides for endosulfan and lindane (Sigma- Aldrich, Germany).

All reagents and chemicals used in this study were of analytical grade.

2.3 Sample Collection

The cowpea sample was obtained from the local market at Lagos street (Ovia North East Local Government Area), Benin city, Edo State. A large batch of cowpea sample was obtained and carefully handpicked to remove dirt and stones before storing them in the refrigerator at the Pesticide Research Laboratory of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria until analysis.

2.4 Preliminary Evaluation

200 g of cowpea was then weighed and pulverized using an electronic blender to obtain fine powder weighing 105.2874 g. The powder was placed in a thimble and then subjected to Soxhlet extraction with 200 ml of dichloromethane as the extracting solvent at 60°C for 1 hour. The extract was filtered into a beaker and allowed to evaporate at room temperature to about 2 ml (approximate size of the sample tube). The extract was cleaned up by passing it through a column using silica gel and sodium sulphate. Thereafter, the extract was then transferred to a sample tube for subsequent analysis.

2.5 Procedure for Analysis

Five groups were created for analysis as follows:

Group 1: Control group with baseline values of lindane and endosulfan

Group 2: Cowpea impregnated with 5% lindane

Group 3: Cowpea impregnated with 10% lindane

Group 4: Cowpea impregnated with 5% endosulfan

Group 5: Cowpea impregnated with 10% endosulfan

For each group the following procedures were carried out:

Pesticide Preparation

i. 5% and 10% Lindane were prepared by weighing 5 g and 10 g of the lindane powder respectively into beakers and 5 ml of dichloromethane was used to dissolve the powder. The solution was made up to 100ml volume with dichloromethane.

ii. 5% and 10% endosulfan were prepared by measuring 12.5 ml and 25 ml of endosulfan respectively into a measuring cylinder and making it up to 100 ml with dichloromethane.

2.6 Sample Preparation

100 g of cowpea was weighed into six beakers and the prepared solutions of the various concentrations of both pesticides were added corresponding to the 5 groups and stirred for about a minute. Thereafter, the beaker was covered with a thin foil paper and the cowpea was left to absorb the pesticides for 24 hours. The treated cowpea for each group was stored at 4°C until ready for use.

Experimentation/Decontamination

This procedure was carried out for 3 sub-groups of each group with stirring done for 0, 15 and 30 minutes respectively.

10% Sodium bicarbonate solution in a beaker was heated to 40°C

i. 10 g of impregnated cowpea was placed in 10% sodium bicarbonate solution, stirred and decanted into a 25 ml beaker immediately.

ii. 10 g of impregnated cowpea was placed in 10% sodium bicarbonate solution, stirred for 15 minutes and decanted into a 25 ml beaker.

iii. 10 g of impregnated cowpea was placed in 10% sodium bicarbonate solution, stirred for 30 minutes and decanted into a 25 ml beaker.

2.7 Extraction

The cowpea sample for each of the subgroups above was washed with distilled water for 30-45 seconds, drained and oven dried at 60° C. Thereafter, the cowpea for each group was pulverized with an electronic blender, and the powder was weighed into a thimble and then subjected to Soxhlet extraction with 150 ml of dichloromethane as the extracting solvent at 60°C for 1 hour. The extract was filtered into a beaker and allowed to evaporate at room temperature to about 2 ml. The extract was cleaned by running it through an artificial column chromatograph made of silica gel that had been placed in between sodium sulfate.

The extract was then transferred into a sample tube and left to air dry at room temperature. After which, all the samples were sent out for GC-MS analysis.

CHAPTER THREE

3.0 RESULTS

The expected outcome of the GC-MS analysis is as follows;

Cowpea samples exposed to 0 minute of treatment with sodium bicarbonate are expected to have a mean concentration of the pesticide residues (lindane and endosulfan) slightly lower than the baseline mean concentration. This reduction may not be significant.

Cowpea samples exposed to 15 minutes of treatment with sodium bicarbonate are expected to have a mean concentration of the organochlorine pesticides (lindane and endosulfan) significantly lower than that of the mean concentration of the control group.

Cowpea samples exposed to 30 minutes of treatment with sodium bicarbonate are expected to have a highly significant reduction in the mean concentration of the pesticide residues (lindane and endosulfan) compared to the control mean and the mean of samples exposed to 15 minutes of treatment.

CHAPTER FOUR

4.0 DISCUSSION

Several factors including a pesticide's characteristics, formulation, and applied concentration affect how long pesticide residues last on agricultural products.

Permanence can also be affected by elements like light, temperature, plant morphology, and growth factors. (Yigit and Veliogu, 2020). Hydrolysis, microbial degradation, oxidation, penetration, and photo-degradation are some of the mechanisms through which processing methods cause pesticides to degrade. The amount of pesticide residues is also impacted by a number of food processing techniques, including washing with water or other aqueous solutions, peeling, chopping, pickling, and heat treatments. Depending on the location of residue, residence time on food, water solubility of residue, washing temperature, and agents used to increase effectiveness. Application of heat might cause residues to be evaporated or hydrolyzed. The mechanisms for removing pesticides can be described as chemical and physical removal. In the chemical way, pesticides can be partly decomposed in chemical solutions and the degradation products can be further removed by washing (Ling *et al*., 2011).

The processes and conditions used in food cooking are highly varied. The details of time, temperature, degree of moisture loss is important to the quantitative effects on residue levels of pesticides. Rates of degradation and volatilization of residues are increased by the heat involved in cooking (Kaushik *et al.*, 2020)

At 0-minute treatment, it is expected that significant amount of pesticide residues may remain on the cowpeas, however, this will be slightly lower than the baseline mean concentration obtained and may differ significantly. This is because the pesticide residues had less contact time (0 minutes) with the treatment solution (10% sodium bicarbonate solution) as well as less heat exposure time which facilitate the removal of pesticide residues.

At 15 minutes treatment, the pesticide residues may be significantly reduced as there was a higher contact time with the treatment solution and more time for evaporation to occur due to exposure to heat which may facilitate thermal degradation in addition to the alkaline environment that sodium bicarbonate provides.

At 30 minutes treatment, it is expected that the pesticide residues will not be detected or will have the most significant reduction in the mean concentration of these residues due to a longer exposure time to heat and the treatment solution. It may be implied that the removal of pesticide residues will be impacted by time of boiling as well as treatment with sodium bicarbonate; the longer the time of boiling, the more efficient the decontamination process. This is in agreement with Kaushik *et al*., (2020) who inferred that processes involving heat treatment can increase volatilization, hydrolysis or other chemical degradation and thus reduce residue levels in cooked food. The mechanism of Sodium bicarbonate decontamination of pesticide residues is not entirely clear, some studies suggest that it provides a suitable alkaline pH for the removal of these pesticides. Surface pesticide residues were most effectively removed by sodium bicarbonate (baking soda, $NaHCO_3$) solution when compared to either tap water, In a study conducted by Yang *et al*., (2017) sodium bicarbonate was effective at removing residues of the fungicide thiabendazole from apples when washed with it, this was attributed to the ability of thiabendazole to degrade in the presence of baking soda, even without application of heat. In the presence of $NaHCO_3$, pesticide residues can degrade, which assists the physical removal force of washing (Yang *et al*., 2017).

It could be implied that heat facilitates thermal degradation of the pesticide residues while baking soda provides an alkaline environment for their degradation.

The stability of the carbon- halogen link renders the lindane persistent and poisonous in nature, therefore the removal of the six chlorine atoms is the most crucial step in the degradation of lindane (Nagata *et al*., 2007). This suggests a possible mechanism of the decontamination of these pesticides through dechlorination by sodium bicarbonate, however further studies are required to buttress this.

In a study conducted by Odion *et al*., (2021) soaking and washing pesticides in water reduces the mean concentration of these organochlorine pesticides (lindane and endosulfan) but not significantly, this is a physical means of removing some pesticide residues.

Relating these studies to this research one could suggest that treatment of cowpeas with sodium bicarbonate combined with heat treatment will significantly reduce pesticide residues from the cowpeas as a synergistic effect will be employed (Heat and baking soda effect) as heat facilitates the degradation of organochlorines, and an enhanced degradation is further achieved in the presence of sodium bicarbonate.

CHAPTER FIVE

CONCLUSION

Treatment of cowpeas with heated solution of 10% of sodium bicarbonate solution may be very efficient in reducing lindane and endosulfan residues in cowpeas. In addition to this treatment, the time of exposure may play an important role in the decontamination process as longer time corresponds to removal of significant amounts of these pesticides. Therefore, sodium bicarbonate is an available, inexpensive as well as a promising option for the decontamination of cowpeas and potentially other food crops from organochlorine pesticides. Further research however, is needed to fully understand the optimal conditions and mechanisms for using sodium bicarbonate as a decontaminating agent and to determine its potential for use in other crops and in different regions.

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