THE EFFECT OF AGRICULTURE PESTICIDES ON BACTERIAL MICROFLORA OF GARDEN SOIL

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DECLARATION

I hereby declare that the project report was written under the supervision of DR. G. E. ADEBAMI and MRS T. F AKINYANJU and it is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and the list of references provided. This researched project report has not been presented for the award of any degree.

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DATE

CERTIFICATION

This is to certify that this research project titled **"THE EFFECT OF AGRICULTURE PESTICIDES ON BACTERIAL MICROFLORA OF GARDEN SOIL."** was carried out by **OYEYELE Oyedamola Victoria** with Matriculation number **19010101030.** This report meets the requirements governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to the Almighty God, the giver of life, and to my parents, Mr. and Mrs. K. T. Oyeyele for their immense support.

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I am greatly indebted to God, for his marvelous doings, his faithfulness and his unconditional love towards me and for his provision and his Grace over my life.

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ABSTRACT

Pesticides are chemical compounds used to kill pests such as insects, rodents, fungi, and invasive plants. As about 10 to 15% of the production of the world's major crops, such as rice, wheat, maize, and potato, is lost each year owing to pest-induced plant diseases. However, most of these agriculture pesticides have a serious and longtime effects on useful soil microbial flora. In this study, isolation and identification of soil bacteria followed by the determination of the effect of commonly used agriculture pesticides on garden soil bacteria were investigated. Isolation and identification using morphological and biochemical characterizations were carried. Four pesticides: Cypermethrin, Lambda-cyhalothrin, Glyphosate ammonium, and Paraquat dichloride were selected and used for soil treatment. Isolation and determination of the colony forming units at 0 to 9 days at different concentrations of the pesticides were investigated. The colony forming unit at 24 hours of incubation ranged from 54×10^{1} - 11×10^{5} cfu/mL and 44×10^{1} -9×10^{5} cfu/mL for the garden maize and eggplant soil samples respectively. *Bacillus* spp. *Klebsiella* spp. and *Pseudomonas* spp. were the predominant bacteria. For the pesticide's application, the colony forming units at the end of 9 days of incubations ranged from 50×10^{3} – 400×10^{3}, 68×10^{3} – 320×10^{3}, 55×10^{3} – 120×10^{3}, 30×10^{3} – 260×10^{3} for glyphosate, lambda-cyhalothrin, cypermethrin and paraquat dichloride respectively. For glyphosate, the highest was recorded at day 3 (1.0 g/ha) and the lowest at day 9 (2.0 g/ha). For Lambda-cyhalothrin, the highest was recorded at day 3 (400 ml/ha) and the lowest at day 9 (800 ml/ha). For cypermethrin the highest was recorded at day 3 (500 ml/ha) and the lowest at day 9 (1000 ml/ha). For Paraquat dichloride, the highest was recorded at day 3 (1.5 ml/ha) and the lowest at day 9 (3.0 ml/ha). This study showed that the investigated pesticides negatively affect microbial counts and activity in soil, which confirmed previously reported environmental concerns.

Keywords: Agriculture pesticide, Garden soil, Soil bacteria, Bacteria count

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Soil is a dynamic living system and consists of a variety of microflora like bacteria, actinomycetes, fungi, and so on. It is primarily made up of inorganic mineral nutrients, organic materials, and an enormous number of life forms, and it promotes a homeostasis between physical, chemical, and biological elements. (Doran and Safley 1997). Agriculture and food production are based on soil. Related to certain features, its symbolic function as a channel for plant implementation, soil conducts a ratio of other crucial tasks, including facilitating gas exchange, energy flow, nutrient and water flow, pollutant detoxification, and several others (Larson and Pierce 1994). Therefore, maintaining soil biodiversity, including microbial diversity, and sustaining sustained agricultural yields depend on managing soil health.

Diverse agrochemicals are employed in modern agriculture (Jagtap, 2012). Pesticides are paramount agricultural agents used to protect crops from pests. Pesticide exposure from repeated applications distorts the interactions of the soil's microorganisms. (Sarnaik *et al.*, 2006) Pesticides are lethal, bioactive substances that affect the quality of agroecosystems and soil productivity directly or indirectly (Imfeld and Vuilleumier 2012). According to the Food and Agriculture Organization of the United Nations, pesticides include a range of chemicals, including insecticides, fungicides, herbicides, rodenticides, nematicides, plant growth regulators, defoliants, fruit thinning agents, desiccants, agents for preventing the premature fall of fruits, and chemicals applied post-harvest to prevent crop loss during storage or transport (FAO). Today's insecticides are primarily synthetic organic or inorganic substances. A few of the features that can be used to review pesticides include the target pest, chemical composition, soil persistence (half-life), spectrum of activity, mode of entry into the target pest, technique of formulation, toxicity of the active molecule, and volatilization behavior. EPA (2012) b; Zacharia (2011); Anonymous (2000).

However, they only make up about 0.5% (w/w) of the soil mass, microorganisms have a big impact on the mechanisms and content of the soil. In the range of 60 to 80 percent of total soil metabolism, the microorganisms are responsible. A study by (Balasubramanian, 2017) explained the most abundant and diverse living organisms, they are also the tiniest (0.1 mm or less in diameter). Myxomycetes, actinomycetes, cyanobacteria, fungi, yeast, and algae are among this environment. Nearly all of them have the capacity to degrade currently existing natural materials. Microorganisms synthesize organic materials into plant nutrients that are assimilated by plants. Soil organisms make up an enormous component of the entire terrestrial biodiversity.

According to a BBC Research survey, the market for synthetic and biological pesticides was valued at $61.8 billion globally in 2014, and it is estimated to have increased to $83.7 billion by 2019. (Lehr,2014). Current studies have stated that the majority of these pesticides breach the cell walls of soil-dwelling bacteria that are not their targets (non-target) organisms, interfering with their normal metabolism and ultimately causing cell death. Therefore, pesticides are recognized as a major threat to soil microbiota and soil health, disrupting the natural habitats in the soil (Sattler et al.,2006). However, despite the numerous efforts aimed at understanding the effect of pesticides on soil ecosystem, it is difficult to comprehend the significance of pesticides in perturbing soil environment due to divergent research findings reported. (Hussain *et al.*, 2009). Then how do these effects depend on the variation of toxicity in microorganisms?

Significant researches have been made in relation to this question. It has been well studied that pesticides if applied in recommended doses have less effect on soil microbes (Imfield and Vuilleumei, 2012).

1.2 Statement of problem

Soil microorganisms have been greatly disregarded on their contribution to the soil's physical, chemical, and biological properties as the excessive application of pesticide is the aggravating concern in the society. Unfortunately, the impact of these pesticides is targeted against the useful and non-target microflora in the soil. In order to upscale the quality and effect of pesticides on microflora there is need for a thorough examination and study of the specific and right amount of pesticides to be used.

1.3 Justification

Pesticides are preferred chemicals for the destruction of pests and weeds for the enhancement of crop production but as a result, most of these chemicals are toxic to the microbial community in the soil which help in the stability of soil health and function.

1.4 Aims of the study

The aim of this research is to determine the effect of commonly used agriculture pesticides on garden soil bacteria.

1.5 Objectives of the study

i. To isolate and culture bacteria from garden soil samples
ii. To identify the selected isolates using morphological and biochemical characterizations
iii. To apply and determine the effect of selected pesticides on soil bacteria.

CHAPTER TWO

LITERATURE REVIEW

The aftermath of pesticides on garden soil and how it influences the interaction of microflora (bacteria) in the soil has been well estimated and researched. However, some surveys outline the usefulness of pesticides as a necessary evil for modern agriculture and a crucial technique for ensuring the security of the world's food supply. (Prashar and Shah, 2016). But the difficulty of continued usage of these insecticides must not be ignored. The impact of pesticides on soil microorganisms is investigated in this literature review. It also shows how the interaction between the microflora in the soil and these agrochemicals through time adds new dimensions to the evolution of these bacteria.

A study on the impact of pesticides on soil microflora disclosed that pesticides tend to have long persistence in the soil so they are defined to affect the soil microflora environment thereby affecting the soil health and which indirectly, lead to a shift in the population dynamics of the soil microflora. (Prashar and Shah, 2016). Also, researches have investigated that the ability of soil microorganisms to degrade pesticides which results in the degradation products of these pesticides which are also assimilated by microbes in the soil resulting in increased population sizes and activities of microorganisms. (Hussain *et al.,* 2009; Hussain *et al.,* 2007 a; Kumar and Philip, 2006; Siddique *et al.,* 2003; Tyess *et al.,* 2006; Das and Mukherjee, 2000 a; Jana *et al.,* 1998). These recent research studies have given an insight into the adverse effect of those pesticides on soil microflora in garden soil samples. Similarly, (Hussain *et al*.,2009) concluded that understanding the application of pesticides could be helpful in elucidating the risk assessment of pesticides contaminations and its adverse impact on soil microbial diversity, enzymatic activities and biochemical reactions.

An increased focus has been placed on research that surrounds the effect of these pesticides in industrial agriculture because of the prevalence of variations in toxicity while research shows that those effects are variable depending on many biotic and abiotic factors ranging from soil characteristics to crop variety (Prashar and Shah, 2016). Also, (Hussain *et al.,* 2009) suggests that intensive future research based on molecular techniques, contrary to traditional approaches should be used for quantification of net impact of pesticides on microbial soil biology and biochemistry.

Such research could contribute to identifying specific strategies and patterns of utilization that will result in the successful management of pesticides at large.

2.1 Origin of soil microbiology

Boussingault, a French agricultural researcher and farmer, established that legumes could obtain nitrogen from air when cultivated in unheated soil in 1838, introducing soil microbiology as a unique field of soil science. Beijerinck, a Dutch scientist, discovered bacteria from nodules of legume roots fifty years later. A number of studies in the field of soil microbiology have been implemented since then. (Giri *et al*., 2005). Soil is the earth's outer layer, composed mainly of loosely structured layers of materials conjured up of inorganic and organic molecules at various levels of organization. (Tate 1995; Kapoor *et al*., 2002). Microorganisms are divided into five major taxonomic classes: algae, bacteria, fungi, protists, and virus. Their activity and interactions with other microbes, larger species, and soil particles are generally influenced by microhabitat conditions, which can differ greatly even over short intervals. (Wieland *et al.,* 2001). The interior and exterior surfaces of soil aggregates of variable size and compositions perform as microhabitats for soil microorganisms. As a result, soil can be regarded variable in terms of the dispersion of soil particles and organisms. (Beare *et al.*, 1995).

2.2 Soil microorganisms

The soil microbial community performs a variety of functions, including nutrient cycling and carbon (Cs) sequestration, which helps to maintain soil fertility and reduce global warming. (Hemkemeyer *et al.,* 2021). Fresh organic materials and nutrients are anabolized into biomass rather than released by catabolic processes, such as respiration or mineralization, which favours microbial growth. (Schimel and Weintraub, 2003; Manzoni *et al.,* 2012; Mooshaminer *et al.,* 2014).

2.3 Functions of soil elements in soil microbiology

The structural elements of biomolecules include: Hydrogen(H), Oxygen(O), Carbon(C), Nitrogen(N), Phosphorus(P), Sulphur (S), Selenium (Se).

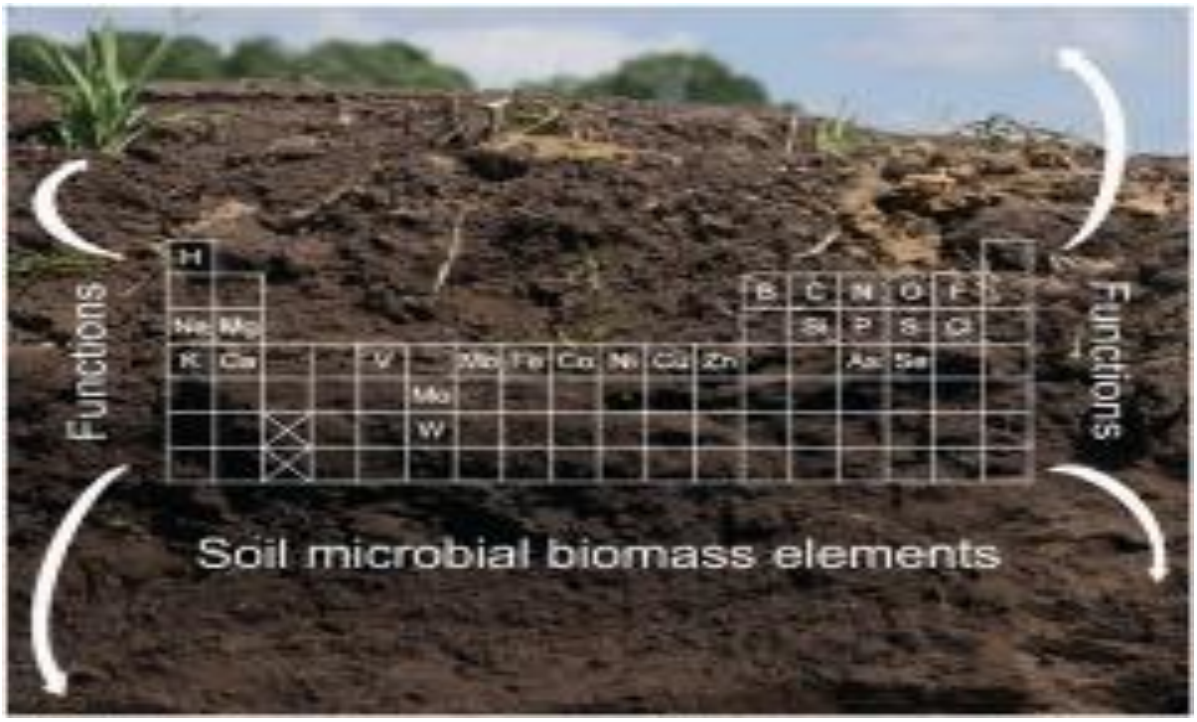

Figure 2.1: Soil microbial biomass elements (Hemkemeyer *et al.*, 2021).

2.3.1 Hydrogen (H) and oxygen (O)

As a result, the elements of this universal solvent, primarily h and o, dominate the elemental composition of organisms. (Williams and Frausto da Silva 1996). In cell energetics, both elements are crucial. They play a role in redox reactions as electron donors (e.g. Corg, h2, nh4+, h2s, h2o), including reduced electron carriers (e.g. NaDH), and electron acceptors (e.g. Co2, no3, so42-, o2), resulting in energy conservation through the creation of a proton (h+) motive force. (Madigan *et al.*, 2019).

Nitrogen from the atmosphere enters the nitrogen cycle via a variety of microbes that can convert N_2 gas to inorganic forms that plants utilize. Nitrogen fixation is the process of converting molecular nitrogen into a form suitable. Because of denitrification, soil erosion, leaching, chemical volatilization, and other factors, nitrogen is the nutritional element most usually reported to be confined to plants. Biological nitrogen fixation (BNF) is therefore significant in agriculture since it provides a source of fixed nitrogen for plant growth that does not require the use of fossil fuels. Diazotrophic bacteria execute biological nitrogen fixation, which is represented by the equation below, in which 2 moles of ammonia are created from 1 mole of nitrogen.

$N_2 + 8H+ + 8e- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16pi$. (Philippot and Germon, 2005).

2.3.2 Carbon (C)

Given the fact that hydrogen and oxygen are essential components of cell building blocks such as lipids, carbohydrates, nucleic acids, and amino acids, the chemical backbone is initiated by carbon (Sterner and Elser, 2002). According to (Kuzyakov and Domanski,2000), all of the organic carbon found in soil are plant derived.

2.3.3 Nitrogen (N)

Nitrogen (N) is an important requirement for all life forms. In terrestrial ecosystems, plant availability of nitrogen is often limited, resulting in intense competition between microbes and plants. (Vitousek and Howarth, 1991). Many microorganisms use glutamate and glutamine as preferred n sources in addition to NH_4, which serve as major n donors for biosynthetic reactions in virtually all cells. (Magasanik, 1993; Merrick and Edwards, 1995; Wong *et al.*, 2008)

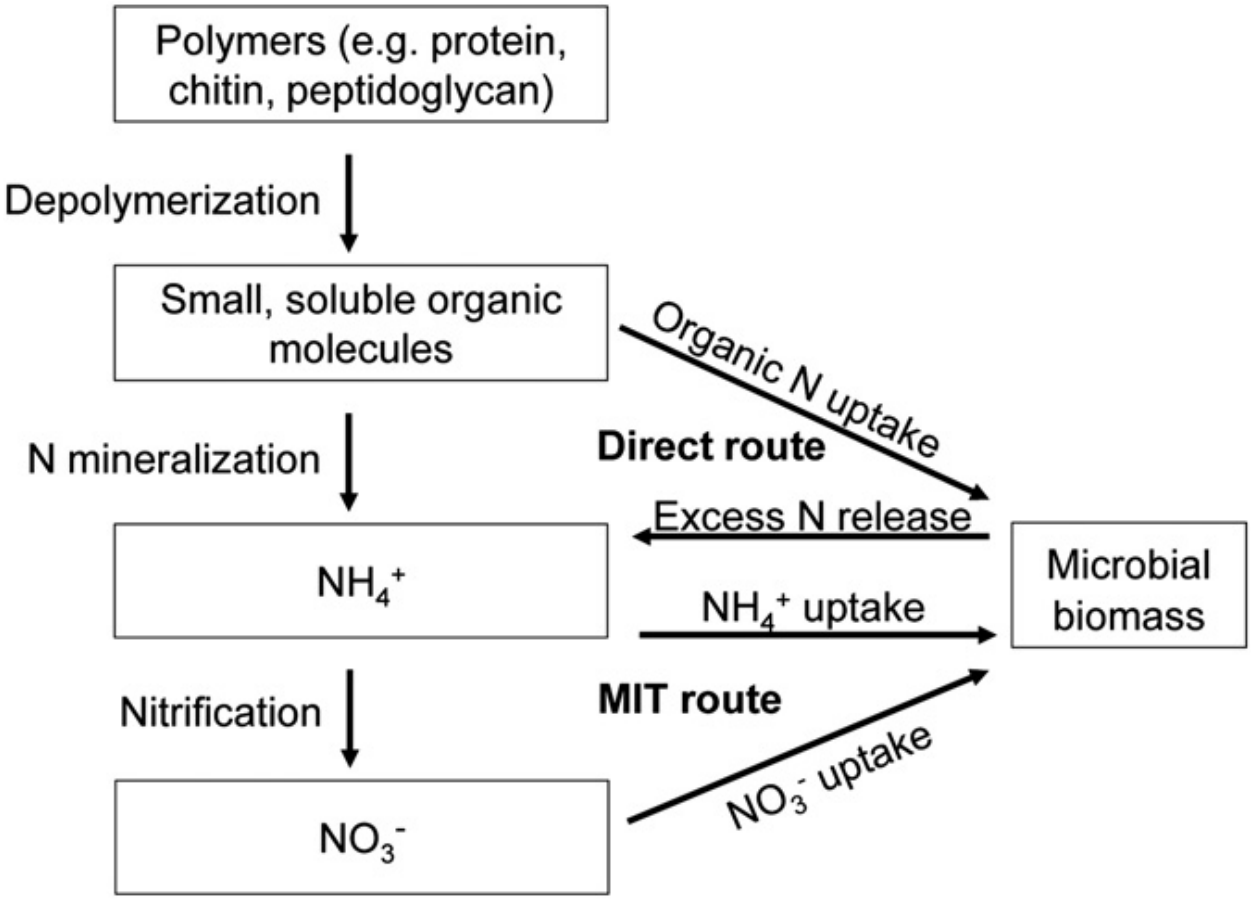

Figure 2.2: Main steps of the microbial n utilization from organic substrates. (Magasanik, 1993; Merrick and Edwards, 1995; Wong *et al.*, 2008).

2.3.4 Phosphorus (P)

Microorganisms play a significant role in mediating the availability of phosphorus (P) to plants in the soil phosphorus (P) cycle. Understanding the microbial contribution to plant P nutrition, as well as the possibility of manipulating certain microbes to increase P availability in soil, has piqued attention for decades. This interest is heightened by the prevalence of P deficiency in weathered and tropical soils around the world, rising p fertilizer costs, and the inefficiency with which plants use p from soil and fertilizer sources, despite many soils containing a relatively large amount of total p that is only sparingly available to plants. (Richardson and Simpson, 2011).

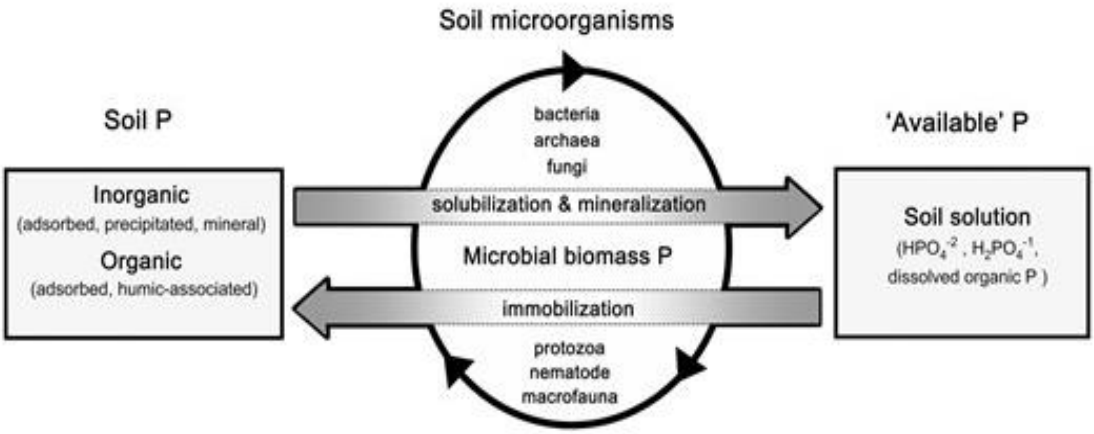

Figure 2.3 Schematic representation of the importance of microorganisms to phosphorus availability in soil. Microorganisms and their interactions in soil play a critical role in mediating the distribution of p between the available pool in soil solution and the total soil p through solubilization and mineralization reactions, and through immobilization of p into microbial biomass and/or formation of sparingly available forms of inorganic and organic soil p. (Richardson and Simpson, 2011).

2.3.5 Sulphur (S)

Sulphur is one of the essential plant nutrients and it that contributes to yield and quality of crops. Sulphur occurs in a wide variety of organic and inorganic combinations. The transfer of Sulphur between the inorganic and organic pool is entirely caused by the activity of the soil biota, particularly the soil microbial biomass, which has the greatest potential for both mineralization and also subsequent transformation of the oxidation state of Sulphur. The Sulphur simultaneously, often referred to as mixotrophic growth oxidizing microorganisms are primarily the gram-negative bacteria currently classified as species of *Thiobacillus*, *Thiomicrospira* and *Thiosphaera*, but heterotrophs, such as some species of *Paracoccus*, Xanthobacter, *Alcaligens* and *pseudomonas* can also exhibit chemo lithotrophic growth on inorganic Sulphur compounds (Vidyalakshmi *et al.*, 2009).

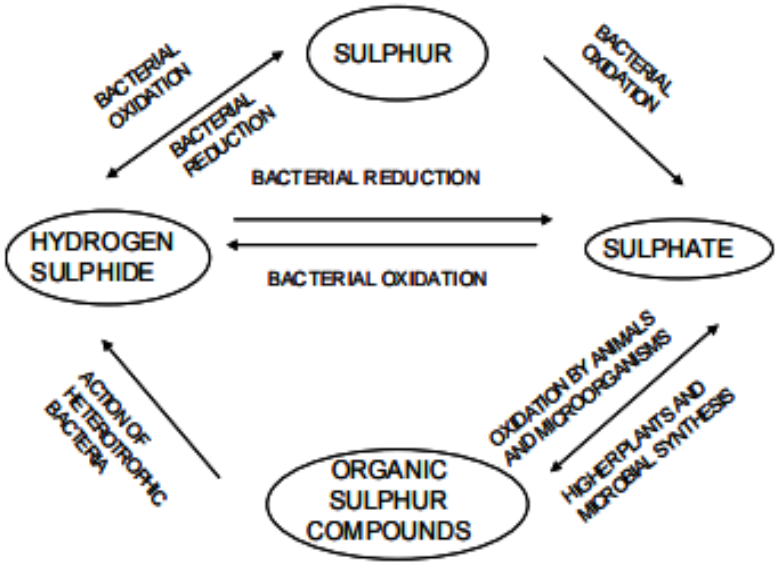

Figure 2.4 The Sulphur cycle (Vidyalakshmi *et al.*, 2009).

2.3.6 Selenium (Se)

Selenium (Se) is an essential trace element for humans and animals and plays a vital role in geochemistry. Soil is a crucial material base for see's entry into the food chain through plants, as one of its major reservoirs. A sufficient level of se in the soil is favorable to plant growth and plays an important role in stress resistance. Among the numerous migration and transformation channels, the transformation of se by microbes is very important, and it is the most common kind of se transformation in the soil environment. (Wang *et al.,* 2021).

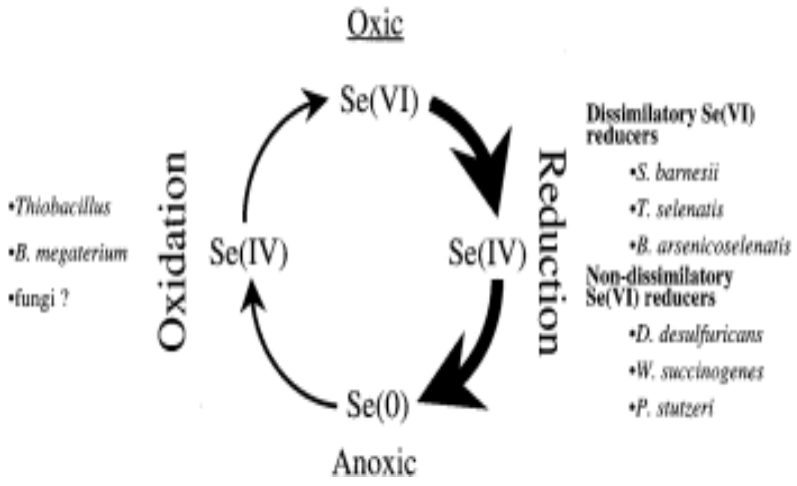

Figure 2.5: Redox cycle of selenium in nature (Dowdle and Oremland, 1998).

2.4 Pesticides

Pesticides and fertilizers are completely reliant on chemicals in modern agriculture. There is no refuting that the much-needed improvement and stability in agricultural productions during the last century was largely achieved by using chemical pesticides and fertilizers to effectively control pathogens and pests, as well as to provide appropriate supplies of vital plant nutrients (Prashar and Shar, 2016). Plant diseases are one of the leading causes of crop loss around the world, posing a significant danger to global food security. Almost 10%–15% of the production of the world's major crops, such as rice, wheat, maize, and potato, is lost each year owing to pest-induced plant diseases (Pinstrup-Andersen 2001). Pesticides are chemical compounds used to kill pests such as insects, rodents, fungi, and invasive plants (weeds).

Certain herbicides are extremely toxic and pose a larger environmental risk than insecticides. As a result, it's important to be clear about which pesticide (together with its dosages and application methods) is being studied in an ecological study. Pesticides are used in world agriculture in a quantity of roughly 2.5 million tons, costing about us$ 16.3 billion each year (Helsel, 1987). Despite the massive use of pesticides and a variety of other pest control technologies, pests manage to kill 36 percent of all potential crops before harvest throughout the world. There is now compelling evidence that some of these compounds are dangerous to humans and other living things, as well as having negative environmental consequences (Forget, 1993). The widespread usage of pesticides in agricultural soils results in harmful chemical pollution (Muoz-Leoz *et al.,* 2013). When pesticides are used, there is a chance that nontarget creatures, such as soil microbes, will be affected (Zhao *et al.,* 2013). Microbes play an essential role in the soil ecosystem (Khan *et al.,* 2010), and their functions in nutrient cycling and decomposition (Lorenzo *et al.,* 2001) are critical (Khan *et al.,* 2007). Pesticides' absorption and persistence in the soil are affected by the soil's composition, pH, and temperature. Plants can digest most organophosphate hydrocarbons, but they can't degrade organochlorinated pesticides, thus they're taken up by them when they're present in soil (Gao *et al*., 2013; Gul and Khan, 2001).

2.5 Types of pesticides

Pesticides are classified into numerous categories based on the pests that are killed or controlled. Fungicides, weedicides/herbicides, nematicides, rodenticides, insecticides, and biopesticides are some of the pesticides available. (Pandya, 2018).

2.5.1 Fungicides

These are chemicals that are used to kill fungal pathogens and eradicate fungal infections on crops. Bordeaux mixture, burgandy mixture, sulphur, mercuric chloride, and other inorganic fungicides are examples. Dithane s-21, dithane m-22, dithane z-78 (all carbamates), oxanthiins (e.g., vitavax), mercury compounds (e.g., agrosan, tillex), and benzimidiazole derivatives are examples of organic fungicides (e.g., benlate). Thiram and Ziram are fungicides, but they are hazardous to zooplankton in water. Antifungal qualities are found in phytochemical extractions such as neem oil, which contains azadirachtin and nimbin. Another example of a fungicide is fentin (Pandya, 2018).

2.5.2 Herbicides

In agriculture, herbicides and weedicides are used to kill undesired plants and weeds. Selective and nonselective herbicides, contact herbicides, translocated herbicides, foliage applied and soil applied herbicides are all available, depending on the method of action. Triazines (e.g., atrazine, simazine), carbamates (e.g., thiocarbamates, phenyl carbamates), and auxin derivatives are all examples of herbicides (e.g., 2,4-d, and 2, 4, 5-t). Agent orange is a combination of 2,4-d and 2,4,5-t that was developed as a defoliant and herbicide during world war ii. Modern herbicides, such as paraquat, have been linked to Parkinson's disease in humans. Herbicide atrazine acts as a teratogen, influencing frog gender development during metamorphosis (Pandya, 2018).

2.5.3 Nematicides

They kill or repel nematodes, for example, aldirab is an acetylcholine esterase inhibitor that is used to fight nematodes that infect tobacco crops in farming. The nematode meloidegyne incognita attacks the roots of tobacco plants, reducing production dramatically. The biological control agent (BCA) purpureocillium lilacinum infests meloidegyne incognita. Proteases and chitinase have been found in one strain of p. Lilacinum, enzymes that could damage a nematode egg shell and

allow a narrow infection peg to pass through. Nematophgus, a carnivorous fungus, may be effective in nematode control. Nematicides include methyl bromide (mb), ethylene dibromide (EDB), and chloropicrin. Soil steam sterilization (SSS) or soil steaming is a technique for disinfecting soil from nematodes and pathogens by inactivating enzymes (Pandya, 2018).

2.5.4 Rodenticides

It is commonly known as rat poison .NA^{+}-fluoroacetate, warfarin, red squill, and zinc phosphide are some examples of rat poison. Rodenticides block the vitamin-k cycle in rodents and mammals, resulting in pest death. In rodents, vitamin d3, d2, and d induce hypercalcemia. Strychnine, a rodenticide derived from the strychnos nux-vomica or semen nut tree, induces suffocation in rats, resulting in death. Chloralose is a chlorinated acetal derivative of glucose that is utilized as a rodenticide as well as an avenicide. Arsenic trioxide, when combined with copper aceatate, makes paris green rodenticide, which is also used as a blue colorant in pyrotechnics (Pandya, 2018).

2.5.5 Algicides

It is a biocide that is used to prevent algae growth in water. As an algicide, cupric sulphate, often known as bluestone, is utilized. Photosynthesis and electron transport chain -inhibitor diuron/dcmu (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is utilized as an algicide. As an algicide, fungicide, and Wormicide/helminthicide/Nematicide, dichlorophen with toluene is employed (Pandya, 2018).

2.5.6 Insecticides

Insecticides are stomach/alimentary canal poisons, contact poisons, or fumigants (inhaled) poisons for insects. *Azadirachta indica* (margosa/neem), *boenighausenia albiflora, peganum harmala*, *derris* (rotenone), and chrysanthemum are examples of natural insecticides (pyrethrum). Larvicide is another usage for *azadirachta indica.* Larvicides include aquabac and vectobac (Pandya, 2018).

On the basis of chemical structure, major pesticides are grouped into: (i) organochlorines, (ii) organophosphates, (iii) carbamates (iv) pyrethroids and (v) triazines.

2.5.6.1 Organochlorines

Organochlorines (OC) are a category of extensively used insecticides that are chlorinated chemicals. These substances fit the definition of persistent organic pollutants (pops) with a high level of environmental persistence. Although OC insecticides were once used successfully to combat typhus and malaria, they are now prohibited in the majority of industrialized nations (Aktar *et al.,* 2009).

2.5.6.2 Organophosphate

Organophosphate pesticides are used extensively worldwide, and poisoning by these agents, particularly in developing nations, is a serious public health problem. The toxicokinetic and toxicodynamic of organophosphate poisoning vary not only with the route and extent of exposure, but also the chemical structure of the agent. The mechanism of toxicity is the inhibition of acetylcholinesterase, resulting in an accumulation of the neurotransmitter acetylcholine and the continued stimulation of acetylcholine receptors (Kwong, 2001).

2.5.6.3 Carbamates

These carbamic acid-derived substances are likely the insecticides with the broadest spectrum of biocidal properties. Since CMS pesticides reduce acetylcholinesterase activity (ache), they can also reversibly inhibit the esterase that causes neuropathy. The two main detoxification processes for carbamate insecticides are hydrolysis and oxidation, with esterase' hydrolysis being the more efficient (Dhouib *et al.,* 2016).

2.5.6.4 Pyrethroids

Pyrethroids are commonly employed as insecticides in both residential and commercial settings, as well as in medicine to treat scabies and head lice on the skin. As part of antimalarial measures, mosquito nets are frequently soaked in solutions of deltamethrin in tropical regions due to their greater sodium channel sensitivity, smaller size, and lower body temperature, insects are approximately 2250 times more poisonous to pyrethroids than mammals are. Mammals are further protected by their slow rate of dermal absorption and quick metabolism of non-toxic metabolites. When combined with either piperonyl butoxide, an organophosphorus insecticide, or both, which

both block pyrethroid metabolism, the mechanisms by which pyrethroids are poisonous alone are already complex. The sodium and chloride channels are the primary targets of pyrethroid actions (Bradberry *et al.*, 2012).

2.5.6.5 Triazines

The triazine herbicides primarily target weed seedlings in the soil. The herbicides can be used on either existing crops or in the relatively short window of time between sowing the crop and its emergence. Triazine herbicides can linger in some soils for many months, and seasonal carryover can occasionally lead to issues. When triazines are applied to soils, their efficacy is influenced by a number of factors, including the soil's structure, organic matter content, moisture content, and particle size distribution. Herbicide persistence can be influenced by how it is applied to the soil; wettable powders and dust formulations are less persistent than granule applications (Dean *et al.*, 1996).

2.6 Paraquat dichloride

Since their introduction as potent nonselective herbicides, the dipyridylium herbicides paraquat and diquat have amassed a wealth of knowledge explaining their phytotoxic mode of action. Although paraquat has been shown to be toxic to nontarget organisms like the soil fauna *collembola*, *acari,* and *homoptera,* as well as other invertebrates and fish, along with experimental laboratory animals and man (Smith and Mayfield, 1977).

Paraquat is a dipyridylium compound. Weed killers are one of the principal applications for dichloride and dimethosulphate. Only when applied on plant leaves are they effective (more so in the light than in the dark), allowing for the careful management necessary to eliminate weeds while protecting nearby taller plants. Additionally, they remove the leaves off crops like cotton and potatoes, making it easier to harvest them using modern methods. They quickly become inactive when they come into touch with soil and are no longer available to plants. Thus, they have a wide range of applications in pasture renewal and can be used to control weeds in a wide range of row crops. Their use can do away with the necessity for ploughing when growing annual crops (Smith and Mayfield, 1977). Weidel and Russo published the first description of paraquat in 1882 and its redox characteristics were identified in 1933 by Michaelis and hill, who gave the substance the

name methyl viologen. Paraquat's herbicidal qualities were initially discussed by (Brian *et al.*, 1958), and it was first made commercially available in 1962. The principal form of paraquat is an aqueous solution with surface-active ingredients. Case studies of patients who ingested paraquat concentrate with the intention of harming themselves are the primary source of information on the clinical course of paraquat poisoning. However, whichever route of absorption is used, the systemic harmful consequences remain the same.

Due to its local gastrointestinal tract irritation, paraquat can cause nausea, which can last for a while, especially after ingesting emetic formulations (Meredith and Vale, 1987). It can also cause vomiting and diarrhea. According to (Vale *et al.*,1987), patients may experience burning, soreness, and discomfort in their mouth, throat, chest, and belly. To try and prevent the particular lung toxicity of paraquat, a wide range of therapeutic medicines have been experimentally examined. Some of the published work is based on single or a small number of cases, while some of it has been applied to humans. In most cases, more than one therapy was used, and knowledge regarding the severity of the poisoning and the initial likelihood of survival is frequently scarce. Due to these factors, it is challenging and frequently impossible to evaluate the effectiveness of any one therapy critically (Lock and Wilks, 2010).

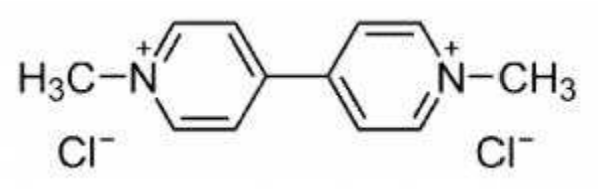

Figure 2.6: i, i'-dimethyl-4,4'-dipyridylium dichloride (Olugbemi, 2013).

2.7 Cypermethrin

A highly powerful synthetic pyrethroid insecticide, cypermethrin is useful for controlling a variety of pests in agriculture, human health, and animal husbandry. Its principal objective in agriculture is to control foliage pests and some surface soil pests, such cutworms, but because it decomposes quickly in soil, it is not recommended to use it to control soil-borne pests that are below the soil's surface. (W.H.O, 1989). Chemically, cypermethrin is the alpha-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropanecarboxylic acid, the dichloro analogue of chrysanthemic acid. Three chiral centers are present in the molecule, two of which are found on the cyclopropane ring and one on the alpha cyano carbon. The four cis- and four trans-isomers of these isomers are typically clustered together, with the cis group being the more potent insecticide. From 50:50 to 40:60, the ratio of cis- to trans-isomers varies. Since cypermethrin is a racemic mixture of all eight isomers, unless otherwise stated, cypermethrin only refers to the racemic mixture (ratio 50:50). (W.H.O, 1989). Although it is likely that photodegradation plays a substantial part in the product's breakdown on leaf surfaces and in surface waters, cypermethrin is rather stable to sunlight compared to natural pyrethrin, and its effects in soils are minimal. Specially in soils rich in clay or organic matter, cypermethrin is particularly highly adsorbed on soil particles. As a result, movement in the soil is very restricted, and under typical use conditions, there is little to no downward leaching of the parent molecule via the soil (W.H.O, 1989).

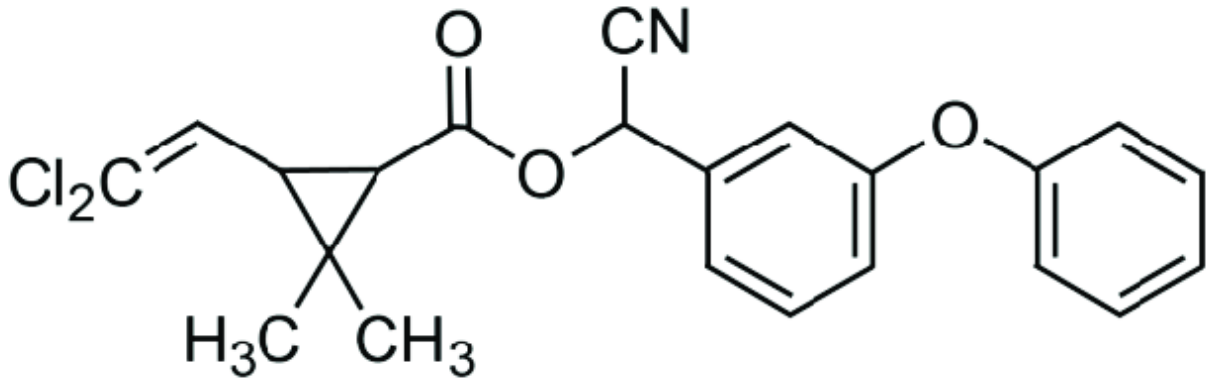

Figure 2.7: Chemical structure of cypermethrin (Georgieva *et al.*, 2021).

2.8 Lambda-cyhalothrin

Lambda-cyhalothrin is a synthetic pyrethroid pesticide and it is used all over the world in agricultural, household pest management, food protection, and disease vector control. Synthetic variants of pyrethrin, which are naturally occurring insecticidal substances produced in chrysanthemum blooms, are known as pyrethroids (*Chrysanthemum cinerariaefolium*). Pyrethroid-containing insecticides have been widely employed to eradicate insect pests in homes, gardens, agriculture, and public health (Amweg and Weston 2005; Oros and Werner, 2005). In agriculture, treatments are applied to control aphid, coleopterous, and lepidopterous pests on cotton, grains, hops, ornamentals, potatoes, and vegetables. Pyrethroids are crucial tools in the management of public health because they can be used to prevent the spread of disease by cockroaches, mosquitoes, ticks, and flies. The suspension of organophosphate products containing chlorpyrifos or diazinon has led to an increase in the usage of pyrethroid products for residential purposes (Oros and Werner, 2005; Weston *et al.* 2005). Risks from exposure to lambda-cyhalothrin can be both initial and long. Skin and eye irritation, non-cardiogenic pulmonary edema, cardiovascular toxicity, convulsions, unconsciousness, and severe muscle fasciculation are some of the acute consequences (Elhalwagy *et al.*, 2015). These chemicals are excellent insecticides for widespread usage in agricultural, veterinary medicine, and public health initiatives due to their high margin of safety for mammals. Finding the potential negative effects that might be connected to the usage of pyrethroids may be urgently necessary given their constantly increasing use (Celik *et al.*, 2003).

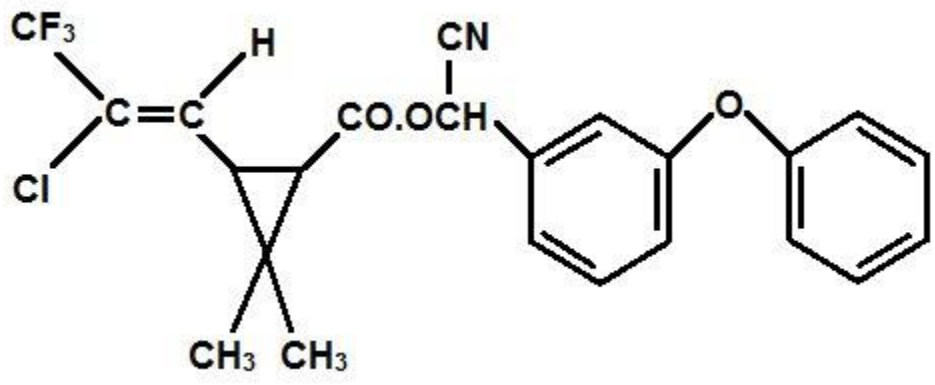

Figure 2.8: Chemical structure of lambda-cyhalothrin (Kanhar *et al.*, 2017).

2.9 Glyphosate

Glyphosate is an unusual herbicide because, with the exception of glyphosine, which has reduced herbicidal effects but exhibits some interesting plant growth regulatory effects, such as promoting sugar cane ripening, virtually no structurally related compounds exhibit any herbicidal activity (Franz, 1985; Hollander and Amrhein, 1980). (Franz, 1985). In 1971, glyphosate's herbicidal characteristics were reported (Baird *et al.,* 1971). The substance was discovered while researchers (Moedritzer and Irani, 1966) were examining the herbicidal properties of tertiary aminomethylphosphonic acids produced from various primary and secondary amines. Only two of the compounds made had any herbicidal action, and both of those compounds had extremely low unit activities.76 of the worst 78 weeds in the world are effectively controlled by glyphosate (Franz, 1985). Its widespread application highlights the herbicide's distinctive qualities. Numerous unique applicators have been created as a result of selective administration to specific plants and the necessity to reduce drift of such an efficient phytotoxin. Recirculating sprayers, rope-wick, roller, and carpet applicators, wet apron hooded sprayers, mist blowers, controlled drop applicators, injectors, and hand-operated sprayers and wipe-on devices are other methods of applying glyphosate in addition to conventional sprayers (Mcwhorter and Derting, 1985).

Glyphosate can be non-harmful to humans and birds but have adverse effect in plants and microorganisms. The formulation, though intentional or inadvertent ingestion of glyphosate is extremely harmful to humans, skin absorption is negligible, and no side effects are anticipated in workers who are adequately protected. Bees, earthworms, and birds are not highly toxic, according to laboratory and field studies of adverse effects on other organisms. Most aquatic organisms were deemed to be at low or negligible risk. While there have been noticeable changes in bird and small animal populations after the use of glyphosate, these changes are linked to modifications in habitat, vegetation cover, and food availability brought on by the herbicide's intended effects (W.H.O 1994).

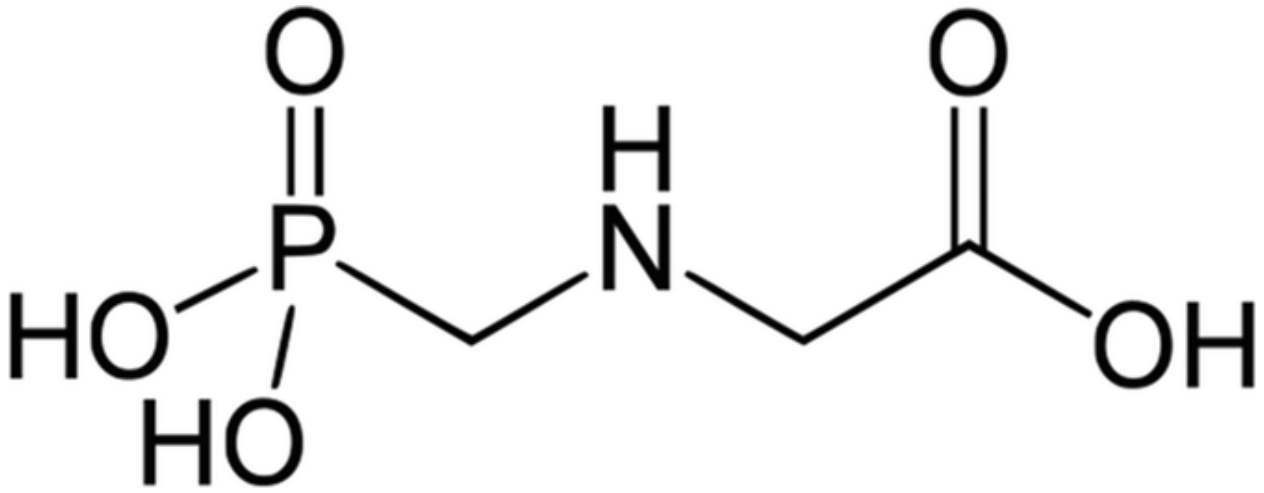

Figure 2.9 Chemical structure of glyphosate (Vaiirlaid *et al.*, 2019).

CHAPTER THREE

METHODOLOGY

3.1 Materials and Equipment

The following materials and equipment were used for this study: distilled water, sterile petri dish, plastic bowls, 1000 ml measuring cylinder, graduated pipette, spatula, 250 ml beaker, 250 ml conical flask, cotton wool, aluminum foil, test tubes, filter paper, McCartney bottles, inoculating loop, glass slides, permanent marker, pesticides (paraquat dichloride, cypermethrin, lambda-cyhalothrin, glyphosate), colony counter, autoclave, hot plates, microscope, incubator, weighing balance, water bath.

3.2 Culture media and Reagent

The culture media used during this experiment is Nutrient Agar. The reagents used during the experiment include: Alcohol (70% ethanol), bromocresol purple, diluted methylene blue, Sodium hydroxide, Grams iodine.

3.3 Collection of soil samples

The experiment was carried out by the collection of soil samples from the school garden (Mountain Top University). The samples were taken to the laboratory for examination and subsequent analysis.

3.4 Soil morphology

The soil sample collected was characterized using: soil type, texture, porosity, color, structure to determine the type of soil to be used for this study.

3.5 Preparation of Culture media

Nutrient agar medium was prepared using 28 g of nutrient agar measured on a weighing balance into a sterile conical flask according to the manufacturer's instruction; 1000 ml of distilled water was dispensed into the conical flask. According to the calculation,7 g of nutrient agar was measured in 250 ml of distilled water. Swirling was done to the solution to ensure proper dissolving

of the medium. The solution is then boiled in the water bath to ensure homogenization after dissolving has been properly done. After boiling, the medium was autoclaved for 15 minutes at 121 ℃. Immediately autoclaving, the medium was gently poured into sterile petri dishes carefully and then allowed to solidify.

3.6 Isolation of soil microorganisms from the soil sample

One gram of the soil sample was taken and serially diluted up to 10^{-6}. From this, an aliquot of 0.1 ml of the 10^{-3} diluted sample was taken and the samples were pipetted evenly over the surface of the plates then, the nutrient agar was gently poured into the plate using the pour plate technique (Athalye *et al.,* 1981). Subsequently, plates were incubated at 37°C for 24 hours and were observed for the appearance of visible colonies. Afterwards, specific colonies were identified and further purified by streak-plate technique and the pure cultures were maintained on nutrient agar (NA) slants at 4°C for storage. Constant sub culturing was carried out to ensure the viability of the isolates.

3.7 Pure culture technique

From the primary plates, different isolates were sub-cultured aseptically by streaking onto the prepared nutrient agar plates. For 24 hours the plates were incubated at 37℃. These resulted in pure culture of the isolated organism. Streaking of the pure culture of isolates was done on a prepared sterile set agar slant in McCartney bottles and kept in the refrigerator for further tests and identification.

3.8 Identification of the Isolates

Further analysis was carried out to determine the identification of the isolates using morphological and biochemical characterization.

3.8.1 Morphological characterization of the isolates

The morphological characteristics of the selected isolate grown on Nutrient agar media was studied for growth rate, colony texture and pigmentation (Promputtha *et al.,* 2005).

3.8.2 Biochemical characterization of the isolates

The selected isolates were further analyzed using these biochemical characterizations: Grams stain, Catalase test, Simmons citrate test, MR/VP tests, Starch hydrolysis test, Sugar fermentation tests (fructose, glucose, galactose, sucrose) for probable identification of the isolates.

3.8.2.1 Grams staining

The Gram stain is fundamental to the phenotypic characterization of bacteria. A smear was made on a glass slide and heat fixed. The crystal violet which is the primary stain was flooded on the fixed culture for 60 seconds; the stain was washed off with water. Iodine solution was added to the smear for 60seconds and was poured off; then was rinsed with water. A few drops of ethyl alcohol (decolorizer) were added and rinsed with water immediately after 5 seconds and finally safranin which is the secondary stain was added for 60 seconds and washed off, then the smear was left to air dry. After the drying of the slide, it was observed under the microscope. Gram staining was done to find reactions of the bacterial isolates to gram reagents. Gram stain helps in distinguishing and classifying bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria.

3.8.2.2 Catalase test

The catalase test is used in differentiating microorganisms which produces the catalase enzyme from microorganisms that do not produce the catalase enzyme. 1 ml of hydrogen peroxide solution is placed in a test tube, and a small amount of bacteria growth was added by wood stick. The formation of air bubbles indicated positive result. (Cheesebrough, 2000). Hydrogen peroxide is converted into oxygen and water by the action of the enzyme catalase. When a little inoculum of a bacterial isolate is added to hydrogen peroxide and the quick construction of oxygen bubbles takes place, it is clear that the enzyme is present. A lack of or poor bubble generation indicates the absence of catalase. The test should not be observed for more than 24 hours.

3.8.2.3 Simmons citrate test

The citrate test evaluates if a bacterial isolate can use citrate as a source of carbon and energy. The synthesis of alkaline waste products from citrate metabolism is the basis for a positive diagnostic test. The color of a pH indicator changes to show how the medium's pH rises as a result. When

identifying gram negative pathogens and environmental isolates, the citrate test is frequently included in a battery of tests. (MacWilliams, 2009). A 2.14 gm aliquot of Simmons citrate agar was dissolved in 500 ml of distilled water gently homogenized using magnetic stirrer while swirling gently to dissolve the medium completely. Afterwards, the medium was sterilized by autoclaving at 121℃ for 15 minutes and allowed to cool at 50℃ and poured in sterile test tubes. The tubes were then stabbed with a loopful of each isolate into each test tube and then transferred to the incubator and incubated at 37℃ for 24 hours. After 24 hours, the tubes were observed. (Olutiola *et al.,* 2000).

3.8.2.4 Methyl red /Voges Proskauer tests (MR/VP)

MR-VP Broth is used for the differentiation of microorganisms on the basis of acid or acetylmethyl carbinol production. 8.5 gm of the MRVP broth was dissolved in 500 ml of distilled water, gently homogenized to dissolve the medium completely. 10ml of the broth was distributed into each test tube, covered with corks and sterilized for 15minustes by autoclaving and then allowed to cool at room temperature. Each isolate was inoculated into each test tube while labeling them accordingly. The tubes were incubated at 37℃ and observed after 24 hours. Afterwards, 5 drops of methyl red solution was added to each solution. The appearance of red color indicates positive reaction while the appearance of a yellow color indicates negative reaction. (Olutiola *et al.*, 2000).

3.8.2.5 Starch hydrolysis test

An organism's capacity to create the extracellular enzymes (exoenzymes) -amylase and oligo1,6-glucosidase that are secreted out of the bacteria and diffuse into the starch agar is tested using the differential medium recognized as starch agar. By severing the glyosidic bonds between the glucose subunits, these enzymes hydrolyze starch, allowing the metabolites to enter the cell. It is also possible to distinguish between members of several genera, such as *Streptococcus, Clostridium, Corynebacterium, Fusobacterium, Enterococcus, Pseudomonas, and Bacillus*, that contain both amylase-positive and amylase-negative species. (Lal and Cheeptham, 2012). An aliquot of 20ml of molten starch agar was aseptically poured into each sterile petri dishes allowed to set and was inverted in an incubator at 37℃. The organism was streaked across the surface of the plate and incubated at 37℃ for 24-48 hours. Afterwards the plates were flooded with some quantity of Gram's Iodine to observed zones of inhibition. (Olutiola *et al.,* 2000).

3.8.2.6 Sugar fermentation test

This test evaluates an organism's capacity to ferment glucose as well as its capacity to transform pyruvic acid, the byproduct of glycolysis, into gaseous byproducts. Gram-negative enteric bacteria, all of which are glucose fermenters but only some of which create gas, are typically identified with this test. A weight of 5 g peptone, 0.5 g of NaCl, 5 g of the fermentable sugar (Fructose, Glucose, Galactose, Sucrose) and a pint of bromocresol purple was measured into a conical flask and the 500 ml of distilled water was added, homogenized, dispensed to 16 test tubes. Inverted Durham tubes were placed in each test tube, covered with corks and sterilized for 15 minutes. Afterwards, each isolate was inoculated into each test tube respectively and incubated at 37°C. After 24 hours, the results were observed for acid and gas production. (Olutiola *et al.,* 2000).

3.9 Preparation and application of selected agriculture pesticide

Four commonly used agriculture pesticides were selected for this study. The pesticides included: Paraquat dichloride, Cypermethrin, Lambda-cyhalothrin and Glyphosate. All the pesticides were prepared and applied according to the manufacturers' instruction.

3.9.1 Paraquat dichloride

Two hundred and seventy-six grams (276 g) of Paraquat dichloride containing 200 g paraquat ion per liter was purchased from Idumota market in Lagos state Nigeria. 1.5, 2.0 and 3.0 liters of the chemical per hectare in 200 liters of water was prepared according to the manufacturer's instruction (AL-Ani *et al.,* 2019). Soil samples from each of the pesticide's concentration were taken at 0, 3, 6, and 9 days and cultured in the laboratory using Nutrient agar plate. Serial dilutions were first prepared and samples from 10^{-3} and 10^{-5} were cultured. Pour plate technique was used for the culturing. Visible colonies were counted at 24 and 48 hours of incubations.

3.9.2 Cypermethrin

Ten percent EC of cypermethrin (Cypermethrin 10% EC) was purchased from Idumota market in Lagos state Nigeria. 500, 800, 1000 liters of the chemical per hectare in 400 liters of water was prepared according to the manufacturer's instruction (AL-Ani *et al.,* 2019). Soil samples from each of the pesticide's concentration were taken at 0, 3, 6, and 9 days and cultured in the laboratory using Nutrient agar plate. Serial dilutions were first prepared and samples from 10^{-3} and 10^{-5} were

cultured. Pour plate technique was used for the culturing. Visible colonies were counted at 24 and 48 hours of incubations.

3.9.3 Lambda-cyhalothrin

Twenty-five grams (25 g, 2.5 EC) of Lambda-cyhalothrin per liter was purchased from Idumota market in Lagos state Nigeria. 400, 600, and 800 liters of the chemical per hectare in 400 liters of water was prepared according to the manufacturer's instruction (AL-Ani *et al.,* 2019). Soil samples from each of the pesticide's concentration were taken at 0, 3, 6, and 9 days and cultured in the laboratory using Nutrient agar plate. Serial dilutions were first prepared and samples from 10^{-3} and 10^{-5} were cultured. Pour plate technique was used for the culturing. Visible colonies were counted at 24 and 48 hours of incubations.

3.9.4 Glyphosate

Glyphosate ammonium 75.7% SG of Glyphosate per gram was purchased from Idumota market in Lagos state Nigeria. 1, 2, and 3 grams of the chemical per hectare in 400 liters of water was prepared according to the manufacturer's instruction (AL-Ani *et al.,* 2019). Soil samples from each of the pesticide's concentration were taken at 0, 3, 6, and 9 days and cultured in the laboratory using Nutrient agar plate. Serial dilutions were first prepared and samples from 10^{-3} and 10^{-5} were cultured. Pour plate technique was used for the culturing. Visible colonies were counted at 24 and 48 hours of incubations.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characterization of the soil sample

Table 4.1 shows the morphology of soil which includes: soil type, color, structure, porosity. The observed soil type is Loamy; the soil color observed is black; the observed structure is granular; and the observed porosity is average. In this present study, the soil was morphologically analyzed according to Table 4.1 and it was concluded that the soil has a high organic matter from the color which was black and with the structure and porosity which has the characteristics of a loamy soil. Loamy soil technically contains between 7% and 25% clay, between 28% and 50% silt, and between 52% and 50% sand, according to a study by AL-Ani *et al.* (2019) which corresponds to the result.

4.2 Colony count from the soil sample

Two different soil sampled were taken from the University garden located in the university premises. The first soil sample was taken from the base of a maize plant and the second sampling was taken from the base of an eggplant. Table 4.2 shows the colony counts of the serial dilution for 10^{-1} ,10^{-3}, and 10^{-5} after the end of 24 hours incubation period. The colony count for these serial dilutions below from the University garden ranged from 11-54, and 9-44 (cfu/mL) respectively. The highest colony count was recorded from the garden maize soil with 10^{-5} serial dilution which shows high microbial activities. According to a study by Yutse *et al.* (2007) who stated that soil microorganisms especially bacteria are physiologically active in moist soil which are commonly used for plant cultivation and also conclude that the soil zone located around active roots has an area of high microbial activity because soil microorganisms creates a food-rich environment for plant growth which supports the results derived from his investigation.

Table 4.1 Shows the characterization of the soil sample

Soil characteristics	Appearance
Soil type	Loamy
Color	Black
Structure	Granular
Porosity	Average

Table 4.2: colony counts of the soil samples

Soil sampling locations	**10^{-1} (cfu/mL)**	**10^{-3} (cfu/mL)**	**10^{-5} (cfu/mL)**
Garden maize soil	540	32×10^{3}	11×10^{5}
Garden eggplant soil	440	12×10^{3}	9×10^{5}

4.3 Characterization of the bacterial isolates

4.3.1 Morphological characterization of the isolates

From table 4.3, the isolates were identified based on their shape, size, color, opacity, elevation, surface, edge, texture. The observed shape of the isolates includes: circular, irregular, rhizoid; the size observed were small (1-2 mm) and medium (3-4 mm). The isolates were opaque/white and milky in color and the opacity observed include: opaque, translucent; elevation observed includes: flat, raised; the surface observed includes: dull/rough, smooth/glistering; the edge observed includes: entire, lobate, the texture observed includes: butyrous, friable. Isolate MGES04 was observed to be translucent in the presence of light. In this study, 9 bacteria were isolated from the garden maize and eggplant soil samples. The morphological characterization of the selected isolates in this investigation was similar to Al-Ani *et al.* (2019) report which shows that the isolates were creamy, and white with a regular and irregular colony shape.

4.3.2 Biochemical characterization of the isolates

Table 4.4 shows the results from the biochemical analysis of the isolates. The biochemical characteristics included: Grams staining, Catalase test, Simmons citrate test, Starch hydrolysis test, Methyl red/Voges Proskauer tests, and Sugar fermentation test (Fructose, glucose, galactose, sucrose). Both positive and negative results of each isolates were observed to give a probable identity of the organism. The tables also include the shape and cell arrangement of the isolates under a microscope. The shape of each isolate was rod ranging from clusters, chains, and single in the cell arrangements. Based on the table, isolate MGES03 is identified to be *Bacillus cereus.* The biochemical characterization of the selected isolates was similar to AL-Ani *et al.* (2019) report which confirms that the garden soil contains *Bacillus sp., Pseudomonas sp., Klebsiella sp.*, as it is to be seen in soil samples for the nourishment of the plant.

Table 4.3 Morphological characteristics of the isolates

Isolates	Shape	Size	Color on NA	Opacity	Elevation	Surface	Edge	Texture
MGES01	Circular	Medium	Opaque	Opaque	Flat	Smooth/ glistering	Entire	Butyrous
MGES02	Circular	Small	White	Opaque	Raised	Dull/ smooth	Entire	Friable
MGES03	Rhizoid	Motile	White	Opaque	Flat	Dull/ rough	Lobate	Friable
MGES04	Circular	Medium	Milky	Translucent	Flat	Smooth/ glistering	Entire	Butyrous
MGMS01	Spindle	Medium	Cream	Opaque	Flat	Rough/ dull	Undulate	Friable
MGMS02	Irregular	Small	White	Opaque	Raised	Smooth/ glistering	Filamentous	Butyrous
MGMS03	Circular	Medium	Milky	Translucent	Flat	Dull/ smooth	Erose	Butyrous
MGMS04	Circular	Medium	White	Opaque	Flat	Smooth/ glistering	Entire	Butyrous
MGMS05	Circular	Medium	cream	Opaque	Raised	Dull/ rough	Entire	Friable

Table 4.4 Biochemical characterization of the isolates

Isolates	Grams staining	Catalase	Citrate	Methyl red	VP test	Starch	Fructose fermentation	Glucose fermentation	Galactose fermentation	Sucrose fermentation	Shape	Cell arrangement	Probable Identity
MGES01	+	+	-	+	-	-	-	-	-	-	Rod	Clustered	*Bacillus sp.*
MGES02	+	+	-	+	-	+	-	-	-	-	Rod	Single	*Bacillus sp.*
MGES03	+	+	+	-	+	+	+	+	-	-	Rod	Chains	*Bacillus cereus*
MGES04	-	+	+	-	-	+	-	-	-	-	Rod	Single	*Pseudomonas sp.*
MGMS01	-	+	+	-	+	+	-	+	-	-	Rod	Single	*Klebsiella sp.*
MGMS02	-	+	+	-	+	+	-	+	-	+	Rod	Chains	*Klebsiella sp.*
MGMS03	-	+	+	-	+	+	-	+	-	+	Rod	Single	*Klebsiella sp.*
MGMS04	+	+	+	-	-	+	-	-	-	-	Rod	Clustered	*Bacillus sp.*

Key: + =Positive, - =Negative, VP= Voges Proskauer

4.4 Pesticide application and colony count

Table 4.5 shows the visible colony forming unit when glyphosate was added to the garden soil sample at 1.0, 2.0, 3.0 g/ha concentrations for 0 - 9 days of incubation. At 1.0 g/ha concentration, the colony forming unit ranged from $84x10^3$ - $400\ x10^3$ cfu/mL. At 2.0 g/ha concentration, the colony forming unit ranged from $63x10^3$ - $160\ x10^3$ cfu/ml and at 3.0 g/ha concentration, the colony forming unit ranged from $50x10^3$ - $260\ x10^3$ cfu/mL. The highest counts for each of the concentration was recorded on day 3 while the lowest was recorded on day 9. The addition of Glyphosate (Glyphosate ammonium 75.7% SG) in Table 4.5 at the recommended, farmer's dosage lowered the number of bacteria by 26% and 33% respectively, during the first hour of incubation. At the ninth week of incubation, the bacteria count had decreased by 17% and 29%, respectively. Just between the sixth and ninth day, this depression was significant. These findings are in line with those of Newman *et al.* (2016) and Aralujo *et al.* (2003), who found that glyphosate reduced the population of bacteria, microbial biomass, and actinobacteria.

Table 4.6 shows the visible colony forming unit when Lambda-cyhalothrin was added to the garden soil sample at 400, 600, 800 ml/ha concentrations for 0 - 9 days of incubation. At 400 ml/ha concentration, the colony forming unit ranged from $90x10^3$ - $320x10^3$ cfu/mL. At 600 ml/ha concentration, the colony forming unit ranged from $71x10^3$ - $260\ x10^3$ cfu/ml and at 800 ml/ha concentration, the colony forming unit ranged from $68x10^3$ - $102\ x10^3$ cfu/mL. The highest counts for each of the concentration was recorded on day 3 while the lowest was recorded on day 9. According to Table 4.6's findings, less bacteria were present when lambda-cyhalothrin pesticide was present. The addition of lambda-cyhalothrin at 400 ml, 600 ml, and 800 ml enhanced the number of bacteria in the first hour and third day of the incubation period, but significantly decreased the number of bacteria at 15% and 24% while at the sixth and ninth weeks of the incubation period, respectively. This report supports the study of Gupta and Baruah (2015), who concluded that the laboratory data indicated a steady decline in the organism's development and pigment content as lambda cyhalothrin concentrations increased in a time-dependent way.

Table 4.7 shows the visible colony forming unit when Cypermethrin was added to the garden soil sample at 500, 800, 1000 ml/ha concentrations for 0 - 9 days of incubation. At 500 ml/ha concentration, the colony forming unit ranged from $75x10^3$ - $120\ x10^3$ cfu/mL. At 800 ml/ha

concentration, the colony forming unit ranged from $62x10^3$ - 90 $x10^3$ cfu/mL and at 1000 ml/ha concentration, the colony forming unit ranged from $55x10^3$ - 100 $x10^3$ cfu/mL. The highest counts for each of the concentration was recorded on day 3 while the lowest was recorded on day 9. Contrary to the results of Tallur *et al.* (2008), He discovered that Cypermethrin serves as many soil bacteria' sole source of carbon, making it susceptible to degradation thereby increasing the population growth of bacteria. Also, when the insecticide cypermethrin was applied, more Gram-negative bacteria were found, according to Zhang *et al.* (2008). Also, contrary to some results, the breakdown of some pesticides increases the availability of plant nutrients like nitrogen in the soil, which helps to increase crop yield. For instance, Glover-Amengor and Tetteh (2008) reported that the yield of vegetable crops that were not adequately treated was higher than the yield of crops that had been treated with lindane under comparable conditions, and that soil degradation caused N to be released, increasing the concentration of N in the soil.

Table 4.8 shows the visible colony forming unit when Paraquat dichloride was added to the garden soil sample at 1.5, 2.0, 3.0 ml/ha concentrations for 0 - 9 days of incubation. At 1.5 ml/ha concentration, the colony forming unit ranged from $30x10^3$ - 177 $x10^3$ cfu/mL. At 2.0 ml/ha concentration, the colony forming unit ranged from $39x10^3$ - 200 $x10^3$ cfu/mL and at 3.0 ml/ha concentration, the colony forming unit ranged from $56x10^3$ - 260 $x10^3$ cfu/mL. The highest counts for each of the concentration was recorded on day 3 while the lowest was recorded on day 9. Table 4.8 of this study demonstrates that the presence of the pesticide paraquat dichloride decreased the bacterial count at all concentrations and during the sixth and ninth day of incubation. The bacterial population was reduced by 30% and 33%, respectively, by the addition of paraquat dichloride at concentrations of 1.5 ml, 2 ml, and 3 ml. A study by Stanley *et al.* (2013) corresponds to this study which concluded that, the herbicide paraquat dichloride applied at both the recommended and 0.5x recommended rates significantly decreased soil bacterial population, diversity, and distribution.

It has been previously studied that the quality of the organic matter in soil has rapidly declined as a result of the application or widespread use of pesticides; which has an impact on the diversity of the microbial flora and fauna (Kalia and Gosal, 2011). By interfering with critical functions including respiration, photosynthesis, and biosynthetic reactions, as well as cell growth and division and molecular composition, pesticides impair nontarget bacteria (DeLorenzo *et al.*, 2001). In soil samples taken from sections with a rice-wheat cropping system, Gupta *et al.* (2000) showed

a detrimental impact of pesticide application on all soil microorganisms with a decrease in the average population of all groups tested. Multiple studies have shown how pesticide toxicity has a negative impact on soil microbial populations. For instance, high quantities of the insecticide glyphosate shifted the dominant bacteria in the soil while also reducing the overall bacterial population in the soil (Moghaddam *et al.*, 2011). Similar findings were made by Goswami *et al.* (2013) and Wesley *et al.* (2017), who concluded that the decline in soil microbial count and biomass can be ascribed to Cypermethrin's harmful effect on soil microorganisms. Cypermethrin and Lambda-cyhalothrin significantly reduced ammonifying, nitrifying, and denitrifying bacteria compared to the untreated sample and impeded the metabolic process (Filimon *et al.*, 2015).

Table 4.5 Colony forming unit (cfu/ml) of glyphosate pesticide at different incubation periods

Pesticides Conc. (g/ha)	Incubation period of pesticide application/colony forming unit (cfu/mL) at 10^{-3} dilution factor			
	0 day (10^3)	3 days (10^3)	6 days (10^3)	9 days (10^3)
1.0	202	400	102	84
2.0	63	160	95	70
3.0	100	260	80	50
Control	confluent	confluent	confluent	confluent

Key: Control- no pesticide added

Table 4.6: Colony forming unit (cfu/ml) of lambda-cyhalothrin pesticide at different incubation periods.

Pesticides Conc. (ml/ha)	Incubation period of pesticide application/colony forming unit (cfu/mL) at 10^{-3} dilution factor			
	0 day (10^3)	3 days (10^3)	6 days (10^3)	9 days (10^3)
400	98	320	120	90
600	90	260	115	71
800	78	confluent	102	68
Control	confluent	confluent	confluent	confluent

Key: Control- no pesticide added

Table 4.7: Colony forming unit (cfu/ml) of cypermethrin pesticide at different incubation periods.

Pesticides Conc. (ml/ha)	Incubation period of pesticide application/colony forming unit (cfu/mL) at 10^{-3} dilution factor			
	0 day (10^3)	3 days (10^3)	6 days (10^3)	9 days (10^3)
500	120	confluent	100	75
800	73	confluent	90	62
1000	100	confluent	81	55
Control	confluent	confluent	confluent	confluent

Key: Control- no pesticide added

Table 4.8: Colony forming unit (cfu/ml) of Paraquat dichloride pesticide at different incubation periods.

Pesticides Conc. (ml/ha)	Incubation period of pesticide application/colony forming unit (cfu/mL) at 10^{-3} dilution factor			
	0 day (10^3)	3 days (10^3)	6 days (10^3)	9 days (10^3)
1.5	30	177	100	83
2.0	39	200	84	47
3.0	90	260	70	56
Control	confluent	confluent	confluent	confluent

Key: Control- no pesticide added

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, garden soil samples were cultured for the isolation and identification of soil microorganisms and to study the effect of the selected agriculture pesticide on the bacterial growth in the soil. Among the four selected commonly used pesticide used for this study, cypermethrin had the most significant the highest and lowest effect on the bacterial during the third and the ninth day respectively and glyphosate having the most minimal effect on bacterial count. With these results, both proven and proposed shows that, the addition of pesticides, (Paraquat dichloride, cypermethrin, lambda-cyhalothrin and glyphosate) drastically reduced the population growth of bacteria.

5.2 RECOMMENDATION

Based on this research, the following recommendations were made

- The creation of new forms of pesticides, including organic and Nano pesticides, as well as the reformulation of existing pesticides comprise the new generation of pesticides.
- Biopesticide research is still regarded as new and developing. Numerous domains, including product production, formulation, distribution, and commercialization, require in-depth research. Some of the biopesticides being developed now could end up being better alternatives for chemical pesticides.

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APPENDIX

Table 4.9 Component of the Nutrient agar

Ingredient	Amount (g)
Yeast extract	2
Peptone	5
Sodium chloride (NaCl)	5
Agar agar	15
Beef extract	1
Distilled water	1 L

Composition of Simmons citrate agar

Composition	Amount (g)
Sodium chloride	5.0
Sodium citrate	2.0
Ammonium Dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.2
Bromothymol blue	0.08
Agar	15
Distilled water	1L

Composition of Starch Agar

Composition	Amount (g)
Peptone	5.0
Yeast extract	2.0
Beef extract	1.0
Sodium chloride	5.0
Agar	15
Distilled water	1L
Starch	1%