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Research Article

# Effects of Agrochemicals (Insecticides) on Microbial Population in Soil

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## Abstract

The effect of insecticides on microbial population in soil was investigated for four weeks. Ten kilogram (10 kg) of soil samples in sack bags were treated with three different insecticides (THIONEX, BEST and DDFORCE) at three concentrations (5 ml, 10 ml and 20 ml). The result of the study showed that the bacterial populations increased with the 5 ml and 10 ml concentrations of the insecticides. The bacterial population in DDFORCE-treated soil at concentration of 5 ml gave a total count of $8.4 \times 10^{11}$ cfu/g. The 10ml concentrations of THIONEX and BEST gave a total bacterial count of $8.1 \times 10^{11}$ cfu/g and $7.0 \times 10^{10}$cfu/g respectively, while the untreated soil sample (0 ml) had $3.5 \times 10^{10}$ cfu/g. The fungal population in DDFORCE and THIONEX-treated soil samples at concentrations of 5 ml and 10 ml were observed to be increasing but BEST insecticide caused significant reduction in the number of soil fungi at all concentrations. The study also revealed that some of the insecticides could be toxic to microbial populations at higher concentrations (20 ml). *Bacillus* species, *Micrococcus* species, *Staphylococcus* species, *Corynebacterium* species, *Saccharomyces* species, *Penicillium* species, *Mucor* species and *Rhizopus* species were some of the bacterial and fungal isolates from the study. The result of this study could serve as a useful guide for determining the acceptable concentration of insecticides that can be applied to soil without adverse effect on soil microbial ecology.

***Keywords:*** *Microbial Population; Insecticides; Soil; Agrochemicals*

## Introduction

Nigeria is a densely populated country. About 80% of people obtain their livelihood through agriculture or agricultural-based industries. To meet the demand of this ever-growing population, the agricultural production needs to be increased, to which the use of agrochemicals with an objective to effectively eradicate crop destroyers, becomes imperative. Agrochemicals could be referred to as any chemical used in agriculture or farming including pesticides (insecticides, fungicides, herbicides, molluscicides, rodenticides, and nematicides), chemical/synthetic fertilizers, plant hormones/vitamins [1].

Pesticide is a broad term, covering a range of products that are used to control pests. Various pesticides are known as insecticides, nematicides, fungicides, herbicides, molluscicides i.e., agents primarily effective against insects, nematodes, fungi, weeds, slug pellets rat and mouse respectively [2]. An insecticide is a pesticide used against insects in all developmental forms. They include ovicides, larvicides and adulticides used against the eggs, larvae and adult stages of insects respectively [3]. Insecticides, however, have been used indiscriminately resulting severe environmental contamination and pollution. The residual effects include carcinogenicity, mutagenicity, reproductive toxicity, respiratory and circulatory problems [4].

A serious concern of the agricultural community is the increase of these insecticide residues in the environment [5], because the application of these xenobiotics in soil can cause damage to the ecosystem and due to the fact that most of these chemicals are stable and can accumulate, they may adversely influence the microbial processes that are an essential part of the Carbon, Nitrogen and Sulphur cycles. Some insecticides can cause a variety of health effects ranging from skin rashes to death. The pathway of attack can arise from intentional or unintentional direct consumption of the insecticides, improper application resulting in the insecticides coming into direct contact with people or wildlife, inhalation of aerial sprays or food consumption prior to the labeled pre-harvest interval. Under extreme conditions, insecticides can also be transported via surface runoff to contaminate distant water source [6].

The role of soil microorganisms in affecting the persistence of agricultural insecticides has gained interest; which is to achieve a rapid disappearance of these highly persistent or toxic insecticides (example, the Organochlorine insecticides) in soil. The degradation of these xenobiotic compounds by soil microflora occurs when the soil microbes use these insecticides as a carbon and energy source [7]. This is also an important means by which these compounds are removed from the environment apart from other mechanisms like biotransformation, photochemical mechanisms, physical mechanisms, chemical and bioremediation of these insecticides contaminated soil. Other roles played by soil microbes include contributing to the aggregation of soil particle, enhancing cycling of nutrients, transforming nutrients from one form to another, assisting plant to obtain nutrients from soil and hindering or assisting water penetration into the soil. The soil being an important recipient and reservoir for the accumulation of these insecticides, an obvious point of concern is what happens to the insecticides in the soil? Does it really cause adverse effects to the soil or vanish immediately? Are these effects permanent or temporary? Ever since the discovery of insecticides, their exploitation and exploration has brought about economic prosperity and helped in nation building.

However, the activities of agrochemicals as a whole in agriculture and other industries have led to environmental pollution and contamination. It is therefore imperative that the environment be decontaminated and reclaimed for human, animal and plant life because some of these compounds, when degraded may be converted to hazardous products in nature that may even be worse than their parent compounds.

## Materials and Methods

### Sample Collection

**Soil Sample:** The soil sample used in this study was collected from a farmland at Nekede, Owerri West, Imo State, on April 14, 2017. The soil sample was collected from 15 cm – 30 cm below the surface of the soil using a shovel. It was transported to the study site in a sterile polyethylene bags. Ten kilograms each was weighed and transferred into nine sack bags and kept in a natural environment (green house) where it can receive sunlight, oxygen, moisture etc.

**Insecticide Sample:** The insecticides used were bought from commercial suppliers in Owerri main Market (Ekeonuwa) in Owerri town. Three (3) different types from different groups of insecticides were used for the study. They include; Thionex (Organochlorine), DDforce (Organophosphates) and Best (Synthetic Pyrethroid). They were used in different concentration to contaminate the soil sample (Table 1).

### Sample Description

### Insecticides Sample

**Ddforce:** This is an organophosphate insecticides used for the control of agricultural, industrial and domestic pest. It is a contact stomach poison acting as a fumigant insecticide. It is used on vegetables, peanuts, cotton, fruit trees to control insect pests of the orders Lepidoptera, coleopteran, dipteral, hemisphere etc. This insecticide contains 1000 g/I dichlorvos in the DDVP.

**Thionex:** This is a broad-spectrum organochlorine insecticides used for the control of pest in cocoa, cotton, soybeans, vegetables and other crops. It controls numerous sucking and biting insect pests such as capsid, thrips, aphids, bollworms, caterpillar, bugs, etc. It is a central nervous system stimulant acting as a contact stomach poison. This insecticide has a long-lasting effect which is speeded up by high temperature and high relative humidity. It contains 350/I endosulfan and aromatic solvent.

**Best:** This is a broad-spectrum synthetic Pyrethroid insecticide, effective for agricultural and horticultural crop insect pests. It is used to control a wide range of insects, especially Lepidoptera on vegetables, potatoes, lettuces, tomatoes, cereals, soybeans, ornamental, forestry etc. It also controls flies and other insects in houses, mosquitoes, cockroaches, houseflies and other insect pests in public health. It contains 10% cypermethrin, solvents and emulsifiers.

**Soil Sample:** The soil sample is a dark brown silt loam soil which is collected from a farmland that has no history of insecticide application. Plant materials and other debris were removed from the soil sample manually before use.

**Experimental Method:** In the preparation of soil sample, Nine (9) sack bags were prepared and each received ten kilograms (10 kg) of non-contaminated soil. These were contaminated with different concentrations (5 ml, 10 ml and 20 ml) of insecticides as shown in Table 1 below. Non-contaminated soil sample was also collected for analysis as the control.

| Insecticides | Volume (ml) | Weight of soil |
|---|---|---|
| DDFa | 5 | 10 |
| DDFb | 10 | 10 |
| DDFc | 20 | 10 |
| Ha | 5 | 10 |
| THb | 10 | 10 |
| THc | 20 | 10 |
| BSa | 5 | 10 |
| BSb | 10 | 10 |
| BSc | 20 | 10 |
| control | | 10 |

***Table 1:*** *Preparation and contamination of soil sample.*

*Key: DDF: DDForce; TH: Thionex; BS: Best; a, b, c: Different concentrations of the insecticides*

**Preparation and Sterilization of Diluents (Physiological Saline)**

**Monitoring and Sampling**

Soil samples were collected every week and analyzed microbiologically for total colony counts and characterization of heterotrophic bacteria and fungi for a period of four (4) week. 100 ml of tap water was sprinkled on the soil sample and mixed thoroughly in every two (2) days interval to ensure adequate moisture/aeration which is necessary for the microbial degradation of insecticides in soil.

Eight and half grams (8.5 g) of sodium chloride (NaCl) were dissolved in 1000 ml of distilled water. The diluents was dispensed in 90 ml and 9 ml portions into conical flasks and bijou bottles respectively, and sterilized by autoclaving at 1210C for 15 minutes. The physiological saline or diluents were allowed to cool before use [8].

**Preparation and Sterilization of Media**

The media used were prepared according to the manufacturer's description. The media used include nutrient agar and sabouraud dextrose agar. Nutrient agar was used to determine the total heterotrophic bacterial count (THBC), while sabouraud dextrose agar was used to determine total heterotrophic fungal count (THFC).

**Determination of Total Heterotrophic Bacterial and Fungal Counts**

Ten grams (10g) of soil sample (contaminated and uncontaminated) were dispensed in 90ml of physiological saline to give $10^{-1}$ dilution. Further dilutions (10 folds) were made after thorough shaking of the soil. The mixture was diluted until $10^{-8}$ dilutions were obtained.

An aliquot portion (0.1 ml) of the $10^7$ and $10^8$ dilutions were inoculated into freshly prepared surface-dried nutrient agar for bacterial count. The same quantity of $10^3$ and $10^4$ were inoculated into freshly prepared surface-dried sabouraud dextrose agar for fungal count. The inoculums were evenly distributed with a sterile glass rod (dipped into alcohol and allowed to burn off on contact with Bunsen flame) and incubated at $37^0$C for 24 hours for heterotrophic bacterial count and 280C (room temperature) for 48 hours for total heterotrophic fungal count [8,9]. Colonies were formed on the plates after incubation and the total heterotrophic bacterial and fungal counts were counted by dividing the petridish into four quadrants at the reverse surface. Total colony forming units per gram was expressed as (cfu/g).

### Identification/Characterization of Isolates

Isolates were identified based on colonial characterization, microscopic examination and few biochemical tests [10]. Wet mount preparation was also done for the examination of fungi.

## Results and Discussion

The ability of microorganisms to utilize pesticides as their sole energy source was demonstrated by Audus [11]. Abundant reports correlated degradation of a wide range of pesticides with microbial activities and the abilities of numerous species of bacteria and fungi to degrade one or more pesticides [12]. It is recognized that the major pathway of pesticides degradation is microbiological [13]. The activity of the soil microbial biomass is of paramount importance in determining the rate of decomposition of pesticides applied to soil. However, the persistence of a number of pesticides in soil and water for very long periods has been reported. This may be due to either the resistance of the pesticides to microbial degradation or the formation of complex with some component of the environment which is largely resistant to microbial attack [14].

The effects of insecticides on population of microorganisms have received considerable attention. Girvan., *et al*. [15] reported increased bacterial and fungal count in the total and culturable communities in soil with different fertilizer and pesticides applications. Soil microorganisms collectively decompose various xenobiotic compounds and return elements to the mineral state utilized by plants [16].

### Microorganisms Isolated from the Study

Bacterial and fungal species were isolated from soil sample. Their colonial characterization, biochemical test, grams reaction and morphology were observed. The bacterial isolates include; *Bacillus, Corynebacterium, Staphylococcus* and *Micrococcus* species. The fungal isolates were mold and yeast which include; *Rhizopus, Aspergillus, Penicillium, Mucor* and *Saccharomyces* species. The bacterial population of the soil exceeds that of the fungal in both number and types (Tables 2-4) because the soil sample was collected on a warm damp soil where fungi do not grow well [17].

| Sample code | Colony code | Size (mm) | shape | elevation | edge | colour | surface | Consistency |
|---|---|---|---|---|---|---|---|---|
| BSta | T1 | 15 | Irregular | Flat | Serrated | Cream | Dull and dry | Granular |
| | T2 | 3 | Regular | Low convex | Entire | Orange | Shiny and moist | Gummy |
| | T3 | 2 | Regular | Low convex | Entire | Yellow | Shiny and moist | Gummy |
| DDFd | T1 | 15 | Irregular | Flat | Serrated | Cream | Dull and dry | Granular |
| | T2 | 4 | Round | Low convex | Entire | Orange | Shiny and moist | Gummy |
| THb | A1 | 12 | Irregular | Flat | Wavy | Cream | Dull and dry | Granular |
| | A2 | 6 | Round | Flat | Entire | Orange | Moist and shiny | Gummy |
| | A3 | 3 | Round | Low convex | Entire | Yellow | Moist and shiny | Gummy |

***Table 2:*** *Colonial characterization of bacterial isolates.*

| Sample code | Colony type | Size (mm) | Shape | Colour | Elevation | Microscopic appearance | Fungal isolate |
|---|---|---|---|---|---|---|---|
| BSta | P1 | 6 | Irregular | Cream | Low convex | Budding, ellipsoidal | *S. cerevisiae* |
| | P2 | 5 | Irregular | Cream | Low convex | Budding, ellipsoidal in aerial hyphae | *S. cerevisiae* |
| DDFb | C1 | 11 | Irregular | White | Filamentous | Non-septate hyphae with sporangiospores in aerial hyphae | *Rhizopus* specie |
| | C2 | 8 | Round | Black | Filamentous | Septate hyphea, branching mycelia and conidia directly on sterigma | *Aspergillus* specie |
| THb | K1 | 3 | Round | Green | Filamentous | Septate hyphae with aerial hyphae on which conidiospores develops | *Penicillium* specie |
| | K2 | 7 | irregular | White | Flat | Non-septate hyphae with sporangium on aerial spores | *Mucor* specie |

***Table 3:*** *Colonial and microscopic characterization of fungal isolates.*

| Sample Code | Gram Morphology | Oxi | Cat | Ind | Cit | Most Probable Organisms |
|---|---|---|---|---|---|---|
| I1 | +R in chains | - | + | - | $S^+$ | *Bacillus* species |
| I2 | +Cocci in tetrads | - | + | + | $S^+$ | *Micrococcus roseus* |
| I3 | +Cocci in tetrads | - | + | - | + | *Micrococcus luteus* |
| T1 | +Cocci in cluster | - | + | - | - | *Staphylococcus* species |
| T2 | +Cocci in cluster | - | + | + | - | *Staphylococcus* species |
| A1 | +R in chains | - | + | + | $S^+$ | *Bacillus* species |
| A2 | +C in tetrads | - | + | + | + | *Micrococcus* species |
| A3 | +R in tetrads | - | + | - | $S^+$ | *Corynebacterium* species |

***Table 4:*** *Microscopic and biochemical characterization of bacterial isolates.*

***Keys:*** *+: Positive Reaction; -: Negative Reaction; S+: Slow Positive Reaction; R: Rods; C: Chains; Cat: Catalase Test; oxi: Oxidase Test; ind: Indole Test; cit: Citrate Test*

**Description of Results Obtained from First Week of Study**

The microbial population of a garden soil treated with insecticides (Thionex, Best and Ddforce) at different concentration increased remarkably after the week one incubation. This agrees with the ability of soil microorganisms to utilize chemicals (insecticides) as nutrients for their growth and cell multiplication [18,19].

In figure 1, comparing the heterotrophic bacterial count to that of the control, the insecticides have acted as stimulator for the population growth of bacteria except in Thionex (20 ml) and Best. The insecticide (Ddforce) at low concentration (5 ml) had the highest increasing effect on microbial population, which is not in conformity with Pandy and Singh [20] who reported that Ddforce (organophosphate) had an adverse effect on soil microbes. Due to the low concentration of these insecticides, it must have been easily inactivated when contaminated with the soil sample. The phosphoric ion content of the insecticides may have been broken down by either photochemical, hydrolysis or chemical processes of the soil to produce active components, which must have been made available as nutrients source for microbes through microbial immobilization (immobilization; conversion of inorganic compounds to organic compounds for microbial assimilation).

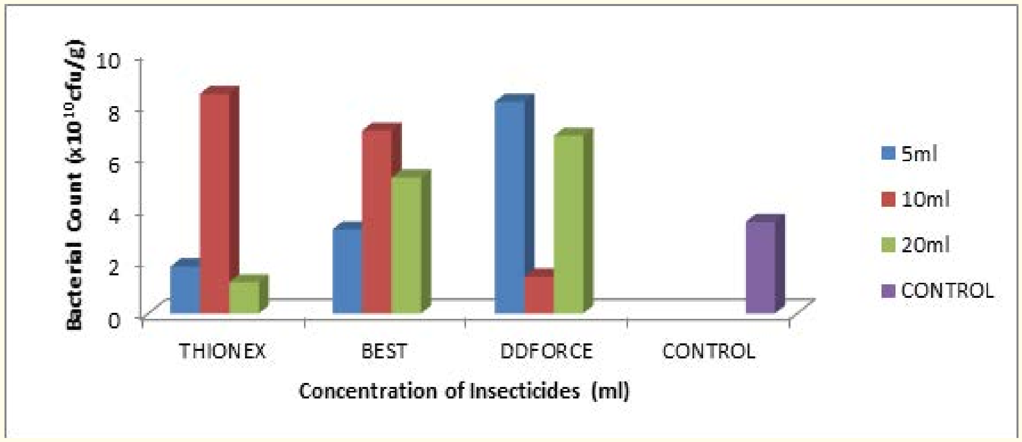


***Figure 1:*** *Comparative representation of total heterotrophic counts for bacterial isolates for week one.*

The insecticide (Thionex) stimulated the microbial growth at lower concentration (10 ml) but at the highest concentration (20 ml) was toxic to them and inhibited their growth. This was supported by the findings of the Digrak and Ozcelik [21] who also confirmed the same result for Thionex (organochlorine).

The insecticide (Best) had a different effect on bacterial population. At lower concentration (5 ml), there was a decreasing effect while at higher concentrations (10 ml and 20 ml), there was an increasing effect. This may have been as a result of the adsorption characteristics of some compounds to the soil which determines how long a compound is bound to soil solids before being inactivated [6]. Due to this adsorption property, the insecticide may not be dissolved in soil water at lower concentration but at higher concentration it may have been dissolved and as such made much available to soil microbes. For fungal count, the insecticides (Ddforce and Thionex), showed an increasing effect on their population when compared to the control. If a synthetic chemical compound is totally different from any that occurs in nature, microorganisms are less likely to have the enzymes necessary for degrading it rapidly [22]. This agrees with the effect of the insecticide (Best) on fungal population as seen in this research which showed a decreasing effect. This does not support the work of Binner., *et al*. [23] who reported that Best (pyrethroid) had no adverse effect on soil fungi.

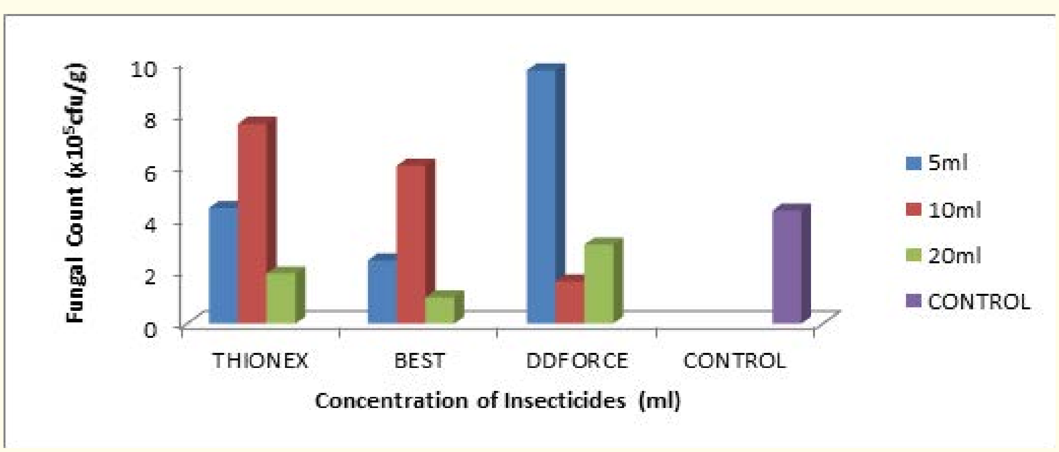


***Figure 2:*** *Comparative representation of total heterotrophic counts for fungal isolates for week one.*

### Description of Result Obtained from Second Week of Study

The analysis of week two result shows that the bacteria population increased only with Best insecticide at low concentration (5 ml) and decreased at higher concentration (10 ml and 20 ml) when compared with the control. The insecticide (Thionex and Ddforce) had an adverse effect on the bacteria population when compared to the population in the control sample. This may be due to the fact that during the first week of study, the population increased because the nutrients were in surplus but in the second week, the microbes released the excess nutrients to plants (mineralization; conversion of organic compound to mineral form for plant utility) and in the process, there may be insufficient nutrient available to them, which reduced their growth and hence decreased effect on bacterial population [24]. Comparing the insecticide-treated soil to that of the control in the fungal population, it was observed that there was general increment in fungal population in the treated soil than in the control which shows that the insecticides supported the fungal growth in soil although it was not much in the soil treated with best-insecticide.

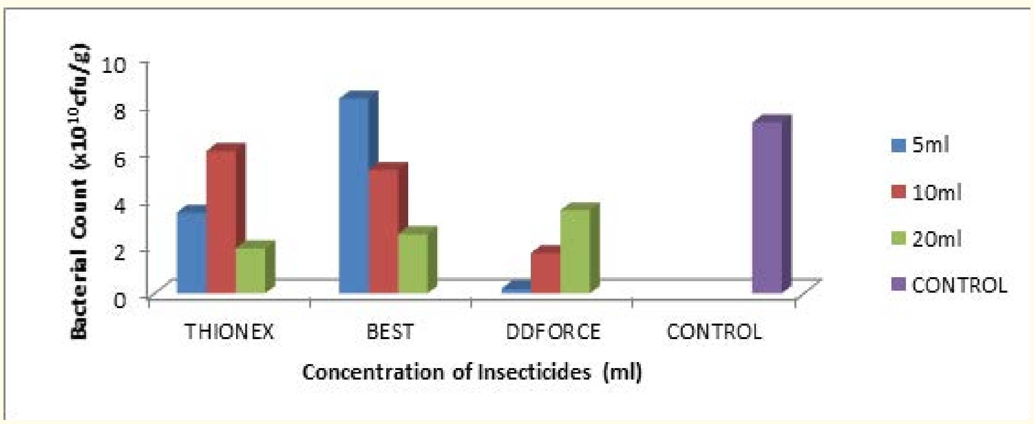


***Figure 3:*** *Comparative representation of total heterotrophic counts for bacterial isolates for week two.*

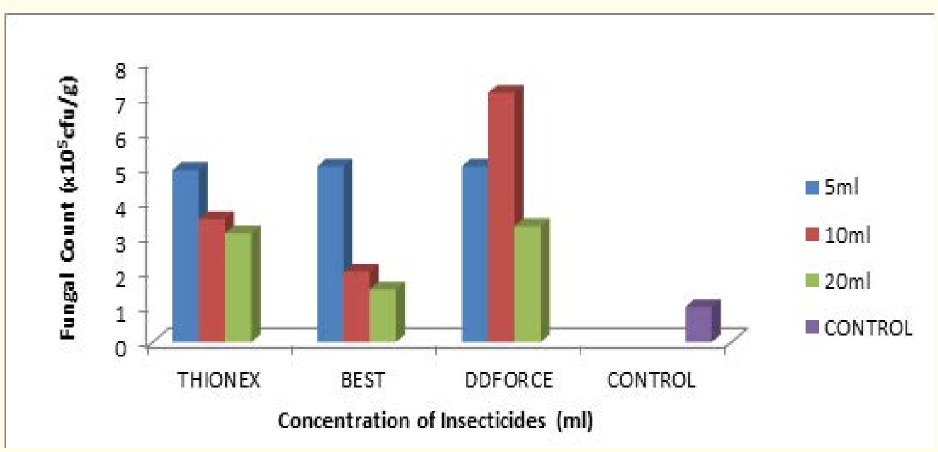


***Figure 4:*** *Comparative representation of total heterotrophic counts fungal isolates for week two.*

### Description of Results Obtained from Third Week of Study

The bacteria population of soil treated with insecticides (Best, Thionex and Ddforce) at different concentrations decreased remarkably after the week three incubation. The moisture condition of the soil for the week seems to change (due to no rainfall) thereby affecting the bacterial population in the soil. This was supported by the principle of limiting factor of microbial ecology which states that ''microbial populations are affected by certain factors called limiting factors which affects the population and its dynamics from time to time and from place to place in response to changes in the environment such as moisture, temperature, pH, etc.'' This also agrees with the analysis done by Lois Bershir [22], that many factors (example moisture, aeration, temperature etc) influence the rate of degradation of compounds by soil microbes. Hundreds of different fungi inhabit the soil. They are most abundant in dry soil where aeration level is more than moisture level [25]. Due to the low moisture condition for the week, the fungal population was able to multiply rapidly, more than the bacterial population. Hence, air and water found in soil pore spaces are directly related and are mutually exclusive.

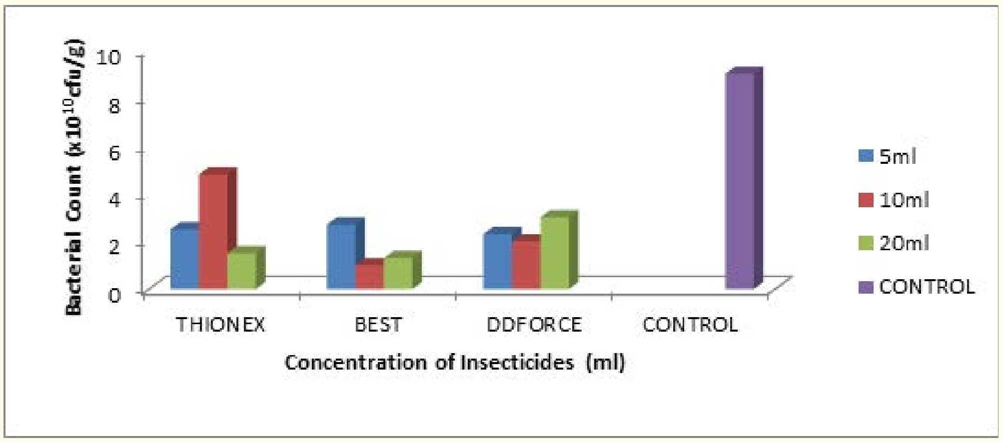


***Figure 5:*** *Comparative representation of total heterotrophic counts for bacterial isolates for week three.*

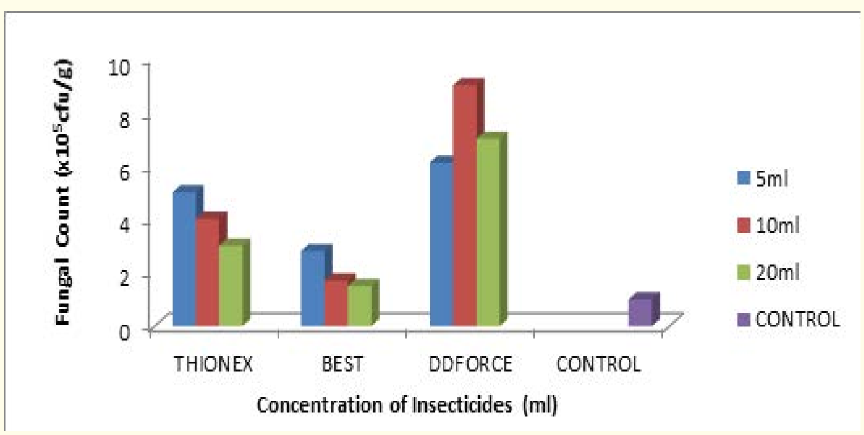


***Figure 6:*** *Comparative representation of total heterotrophic counts for fungal isolates for week three.*

**Description of Results Obtained from Fourth Week of Study**

The result of the fourth week showed that the insecticides (BEST and DDFORCE) had an increasing effect on the bacterial population when compared with the control. The increase was as a result of the microorganisms still utilizing the insecticides as nutrient source for their growth and cell multiplication. This increase was obvious in the highest concentration of the insecticide (BEST: 20 ml) with 9.1 x $10^{11}$ cfu/g over the Control with 7.5 x $10^{11}$ cfu/g. According to Lois Beshir, any practice that favours the multiplication of microorganisms will increase the rate of degradation. Generally, the insecticide (THIONEX) has been observed to be decreasing the bacterial population right from the second week of study to the fourth week. This may be that, the chemical (insecticide) had been toxic to their cells, which could also be referred to as decline as it resulted to reduced bacterial population. The fungal populations increased with all the insecticides (BEST, THIONEX and DDFORCE) compared to the Control sample. The highest fungal count was observed at the insecticides (DDFORCE: 10 ml) with 8.1 x $10^6$ cfu/g over the Control with 1.4 x $10^4$ cfu/g. among the three insecticides applied, BEST proved to be the most destructive (recalcitrant) on soil fungi initially but with further exposure and adaptation of the fungi to the environment, it became susceptible fungal attack. According to Dr. Alexander Marthin of Cornell University, he stated that ''no organic or inorganic compound is immuned to microbial degradation provided that the environmental conditions are available'' [24,26].

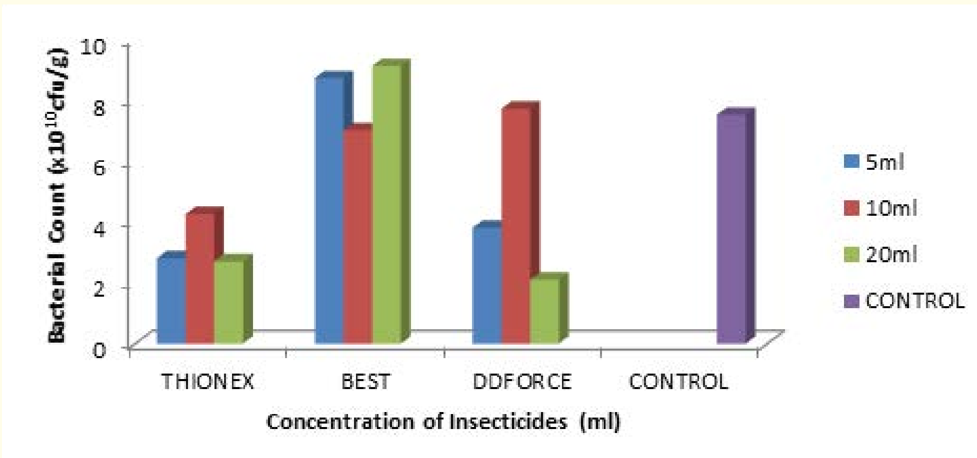


***Figure 7:*** *Comparative representation of total heterotrophic counts for bacterial isolates for week four.*

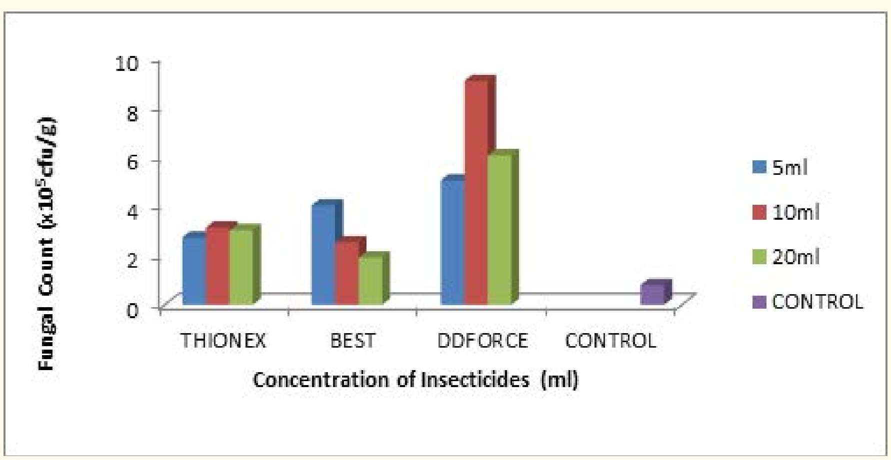


***Figure 8:*** *Comparative representation of total heterotrophic counts for fungal isolates for week four.*

## Conclusion

The results of the present study revealed that the insecticides had positive effect on total number of soil bacteria and fungi but at low concentrations. Comparing the results of the insecticides-treated soil samples to that of the control soil samples, it was observed that at lower concentrations of the insecticides (5 ml and 10 ml) there was an increasing effect on microbial population which must have been that the insecticides were broken down by soil processes and as a result were immobilized by microbes through microbial degradation. At higher concentration (20 ml), a negative effect of the insecticides was observed on microbial population.

This higher concentration must have been toxic to soil microbes and at the same time not easily degraded due to its large amount; hence the decreasing effect observed in the population. It is therefore concluded that insecticides should be safely used at the concentrations of 5 ml and 10 ml since at 20 ml concentrations are toxic to soil microbes.

## Recommendation

Soil microorganisms play important roles in the environment which include decomposition processes, biochemical cycling, energy transfer through trophic levels and numerous microbes-microbes interactions, microbes-plant and microbes-animal interactions, if these functions are impaired, the environment will be unfit for human habitation and because human health goes along with its environmental state; so the environment should be protected. It is therefore, advisable that the use of insecticides be controlled, since at higher concentration they are toxic to soil microbes.

I suggest that all insecticides be treated prior to approval for commercial sale and labeling by the Environmental Protection Agency (EPA), even the approved ones should be used only in the manner specified on the product label. I also suggest that the isolated bacterial species can be employed in the microbe-based bioremediation of insecticides-polluted soil (Bioaugmentation).

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