Impact of Pesticides Application on Soil Microorganisms in a Cucumber (*Cucumis sativus*) Farm

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Abstract

Pesticides are chemicals or substances which is potent enough to kill, repel or prevent any pest. The impact of application of pesticides on soil microorganisms in a cucumber farm was investigated. The soil samples were collected before the chemical was sprayed and 24 hours after spraying. Samples were collected into sterile bags using sterile soil auger at 0-15 cm depth. The samples were collected weekly for four weeks (one month). Standard microbiological techniques were employed using Nutrient and Sabouraud dextrose agar plates in duplicate for enumeration and isolation of bacteria and fungi. The organisms were identified using biochemical methods.The total heterotrophic bacterial counts before addition of pesticides for week 1, 2, 3 and 4 are as follows:4.6×10^5,7.2×10^5, 1.1×10^6 and 1.1×10^6 cfu/g, respectively. The total heterotrophic bacterial counts after spraying the pesticides for weeks 1, 2, 3 and 4 are: 8.6×10^5, 1.9×10^5, $1.8\times10^6$and 1.0×10^6 cfu/g. The heterotrophic fungal counts before addition of pesticides for weeks 1, 2, 3 and 4 are:4.6×10^3, 1.5×10^4, $4.3\times10^4$and 2.9×10^4 cfu/g. The heterotrophic fungal counts 24hours after spraying for weeks 1, 2, 3 and 4 are: 8.7×10^3, 5.8×10^3, $2.4\times10^4$and 8.5×10^3 cfu/g, respectively. The results showed fluctuations in the fungal counts but despite these fluctuations, no significant difference at ($p \leq 0.05$) was observed in the pesticide treated and untreated soils.Seventy-four bacterial isolates which belonged to: Bacillus subtilis, Bacillus sp, Bacillus lentus, Micrococcus sp, Staphylococcus sp, Pseudomonas aeruginosa, Bacillus cereus, Proteus sp, Bacillus mycoides, Flavobacterium sp, Bacillus licheniformis, Escherichia coli and Pseudomonas fluorescens were identified while forty fungal isolates belonging to: Penicillium sp, Saccharomyces sp, Mucor sp, Aspergillus flavus, Aspergillus niger, Microscporium sp, Aspergillus lentulus, Cladophialophora sp, Basidiobolus sp, Paceleomyces sp, Neosatorya sp, Rhizopus sp and Fusarium sp were isolated. The frequency occurrence of Bacillus mycoides, Flavobacterium sp, Bacillus subtilis, Bacillus sp, Bacillus lentus, Micrococcus sp, Staphylococcus sp, P. aeruginosa, Bacillus cereus, Proteus sp, B. licheniformis and Pseudomonas fluorescens were: 14.9, 12.2, 10.8, 10.8, 4.1, 8.1, 5.4, 9.5, 9.5, 1.4, 8.1 and 5.4 %, respectively. Bacillus mycoides was the most predominant bacteria while Flavobacterium sp was the second most predominant bacterial isolates. The frequency occurrence of Penicillium sp, Saccharomyces sp, Mucor sp, Aspergillus flavus, Aspergillus niger, Microscporium sp, Aspergillus lentulus, Cladophialophora sp, Basidiobolus sp, Paceleomyces sp, Neosatorya sp, Rhizopus sp and Fusarium sp were 15, 2.5, 7.5, 5, 20, 5, 2.5, 5, 5, 15, 2.5, 7.5 and 7.5 %, respectively. A. niger was the most predominant fungal isolates. The addition of chemical pesticides resulted in fluctuations in microbial populations and types in the soil samples. This may have detrimental impacts to soil fertility over time. Moreover, accumulation of chemical pesticides in the plants could be a public health problem, as this could predispose consumers' to toxic chemicals.

Keywords: *Pesticide application, Soil Microorganisms, Cucumber farm*

Introduction

In other to maximize food production to meet up the high demand of agricultural products, pesticides are used in agriculture to prevent pest infestations. Pesticides are vital agrochemicals utilized in farms to prevent crops from pest infestation and they are usually applied in the farms at different times during planting season (Sanjay and Divya, 2016). The introduction of chemical pesticides as a means of controlling pests in agriculture offers great benefits especially in the high yield of agricultural products. Despite its numerous benefits, there are concern that the chemicals could be assimilated in the soil thereby altering the microbial diversity of beneficial microorganisms which are known to promote and support plant development in the soil. According to the USEPA (2014), any substance or mixture of substances which could be used to prevent, kill, repel, or control any pest and can also serve as plant regulators, defoliants, or desiccants are known as pesticides.Pollution of the soil results from the application of the substance on the crops. Muñoz- Leoz*et al*. (2013) reported that the extensive consumption of pesticides in cultivated soils leads to the pollution of thesoil with harmful materials. The harmful materials imbedded in pesticides could impact on soil organisms by altering their balance which could affect the fertility of the soil. Previous study has reported that soil fertility does not only depend on soil texture but also on the biological strength within the soil. Thus, the addition of pesticides could alter the microbial diversity either by directly or indirectly affecting their activities which in turn could have a negative impact on the fertility of the soil (Chi-Chulo, 2010).

Soil microorganisms play major roles in improving the fertility of the soil especially in fixing nitrogen and other important minerals in the soil (Prescott *et al.,* 2011).The nature of the pesticide, the concentration applied, physical conditions, chemical and biochemical conditions are the factors that could influence the impact of pesticides on soil microorganisms (Aurelia, 2009; Sethi *et al.,* 2013). Previous studies have illustrated that microorganisms have the ability to grow in the presence of many commercial pesticides and that these microorganisms could detoxify the pesticides as they use them as carbon and energy source (Sanjay and Divya, 2016).

The cucumber is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiraling tendrils (Mariod *et al.,* 2017). The fruit is used in preparing many delicacies in many parts of Rivers State. The cucumber farm is a farm where cucumbers are grown for commercial or subsistent purposes. Many of the farms are sprayed with pesticides to control pest infestation which could cause poor yield during harvest. Despite their benefits, pesticides can be hazardous to both humans and the environment (Fenik *et al.,* 2011). Many pesticides which can remain in the environment for a long time leading to environmental contamination, resulting in biomagnifications and concentration, building up in the food chain to higher trophic levels can expose the general population to pesticides residues, including physical and biological degradation products present in the air, water, and food (Mostafalou and Abdollahi, 2013). The knowledge of soil microbial ability to degrade pesticides and the impact of pesticides on microbial diversity in soil are still limited (Chi-Chulo, 2010). Thus, this study is aimed at providing information on the impact of pesticides on soil microorganisms in a cucumber farm.

Materials and Methods

Collection of Soil Samples

Soil samples from a cucumber farm were collected along the ridges(1m apart) to make composite samples. The farm is on a four plot land fenced, located behind God'scity estate, in Ozuoba, Obio-Akpor Local Government Area of Rivers State. Soil samples were collected with soil auger from 0-15cm depth into a sterile black polythene bags. The samples were collected weekly for a four weeks period.

Pesticide used in the Farm

Pesticide used in this farm is magic force purchased from a chemical shop in Port Harcourt. The chemical was mixed according to manufactures instruction.

Treatment of Farm

The pesticide is sprayed on the young cucumber plant from two weeks after planting, once a week, until harvest. Treatment with pesticide was done weekly to rid the farm from pest infestation which could cause great economic loss to cucumber yield. The impacts of the pesticides on the soil microbes were monitored by collecting soil samples before the pesticide was sprayed and 24hours after the introduction of pesticide in the farm. Thus, soil samples were collected weekly for microbial analysis.

Enumeration and Isolation of Microorganisms

The soil samples were evaluated by determining the total heterotrophic bacterial and fungal counts. Ten-fold serial dilution was carried out by transferring 10g of soil into 90ml sterile normal saline in a 250ml conical flask and this served as the stock. The flask was carefully agitated to dislodge microbes and was allowed to settle. After which, 1ml was withdrawn with a pipette from the stock and transferred into a test tube containing 9ml sterile normal saline. This dilution continued until dilution of 10^{-5} was obtained. Aliquots of 0.1ml from dilutions of 10^{-4} to 10^{-5} for bacteria and 10^{-2} and $10^{-3}$for fungi were inoculated on prepared Nutrient agar (NA) and Sabouraud dextrose agar (SDA) plates in duplicates for enumeration of the total heterotrophic bacteria and fungi present in the respective soils. The inoculated plates were spread evenly using sterile bent glass rod. Nutrient agar plates were incubated at 37°C for 24hours while SDA plates were incubated at 22°C for 3 days (Douglas and Robinson, 2018). After incubation, colonies on the respective plates were counted and used in enumerating the bacterial and fungal counts. Distinct colonies were sub-cultured on prepared NA and SDA plates for bacterial and fungal isolates and incubated.

Characterization and Identification of Bacterial Isolates

The bacterial isolates were identified by their cultural and biochemical characteristics. Cultural characteristics used were; colony morphology (colour, shape, size, texture and elevation) and gram reaction. The biochemical tests employed were; methyl red, Voges-Proskauer, sugar fermentation, indole, oxidase, catalase, citrate utilization and catalase tests. The tests were carried out as described by Cheesbrough (2005). Isolates were further confirmed using the automated biometric identification system (ABIS) online data base and Holt *et al.* (1994).

Identification of Fungal Isolates

Fungal isolates were identified by macroscopic and microscopic examination of cultures. The macroscopic identification is based on colonial morphology, the colour (pigmentation), surface appearance and texture of fungal colonies. While microscopic examination was done using wet mount method, observing their appearance under the microscope (Douglas and Robinson, 2018). In the microscopic examination, spores of fungal isolate were placed on a grease free slide containing drop of lactophenol blue. The slides were covered with a cover

slip and viewed under the microscope using the X10 and X40 objective lens, checking for sporangia, conidia, vegetative mycelium, septate and non-septate hyphae. The characteristics of the fungal isolates viewed were compared with those contained in fungal atlas and book of medical fungi (Sarah *et al.*, 2016).

Statistical Analysis

The means and standard deviation of the counted colonies of bacterial and fungal counts were obtained using GraphPad Prism (version 9). The logarithmic values of the mean and standard deviation were used to plot the graph. The frequency of occurrence of bacterial and fungal isolates was calculated using MS Excel (2019). One-way ANOVA was used to check for significant difference between the samples.

Results

The results of the total heterotrophic bacterial and fungal counts are presented in Figure 1. Results showed variations in both the total heterotrophic bacterial and the fungal counts before and after treatment with the pesticides across the weeks. The total heterotrophic bacterial counts before and after introduction of pesticides in week 1 are $4.6\pm0.39 \times10^5$ and 8.6 ± 0.64 x10^5cfu/g. The total heterotrophic bacterial counts before and after introduction of pesticides in week 2 are $7.2\pm0.59\times10^5$ and $1.9\pm0.40\times10^5$cfu/g. The results of total heterotrophic bacterial counts before and after introduction of pesticide in week 3 are; $1.1\pm0.52 \times10^6$ and$1.8\pm0.51\times10^6$cfu/g. The total heterotrophic bacterial counts before and after introduction of pesticides in week 4 are: $1.1\pm0.5\times10^6$ and $1.0\pm0.5\times10^6$cfu/g. Total heterotrophic bacterial counts fluctuated across the weeks. That is, there was a rise and fall trend as illustrated in Fig 1. While the heterotrophic bacterial counts before introduction of pesticide in week 1 were lower than the heterotrophic bacterial counts after introduction of pesticide in week 1, the heterotrophic bacterial counts in week 2 before introduction of pesticide were higher than the counts obtained after introduction of pesticide. Similarly, in week 3, the heterotrophic bacterial counts before introduction of pesticide were lower than the counts obtained after introduction of pesticide. While the counts obtained in week 4 saw a reduction of bacterial counts from 1.1×10^6 before spray of pesticide to 1.0×10^6cfu/g after spraying the pesticide. With the exception of week 1 and week 3 which showed higher bacterial counts after introduction of pesticide, week 2 and week 4 showed low counts in the heterotrophic bacteria after introduction of pesticide. Thus, the pesticide in one occasion has a negative impact on the bacterial population while in other occasion, it encouraged bacterial growth.

As observed in the bacterial counts of the soil samples, the fungal counts varied also, with less fluctuation as observed in the bacterial counts. The heterotrophic fungal counts before and after introduction of the pesticide in week 1 are $4.6\pm0.1\times10^3$ and $8.7\pm0.3 \times10^3$cfu/g. The heterotrophic fungal counts reduced from $1.5\pm0.2\times10^4$cfu/g before introduction of the pesticide to $5.8\pm0.3\times10^3$cfu/g after introduction of pesticide in week 2. Also, the heterotrophic fungal counts reduced from $4.3\pm0.5\times10^4$ before introduction of pesticide in the farm to $2.4\pm0.4\times10^4$cfu/g after introduction of pesticide. Similar observations were made in week 4 which also had the fungal counts reduced from $2.9\pm1.1\times10^4$cfu/g before introduction of pesticide to $8.5\pm0.47\times10^3$cfu/g after spray of pesticide. This trend is illustrated in Figure 1. Thus, apart from week 1 which showed that the fungal counts were higher before the introduction of pesticide, the trend in the heterotrophic fungal counts showed that the pesticides impacted negatively on the fungal populations by reducing the fungal counts.

Despite the fluctuation in the bacterial and fungal counts, no significant difference ($p \leq 0 .05$) was observed in the pesticide treated and untreated soils.

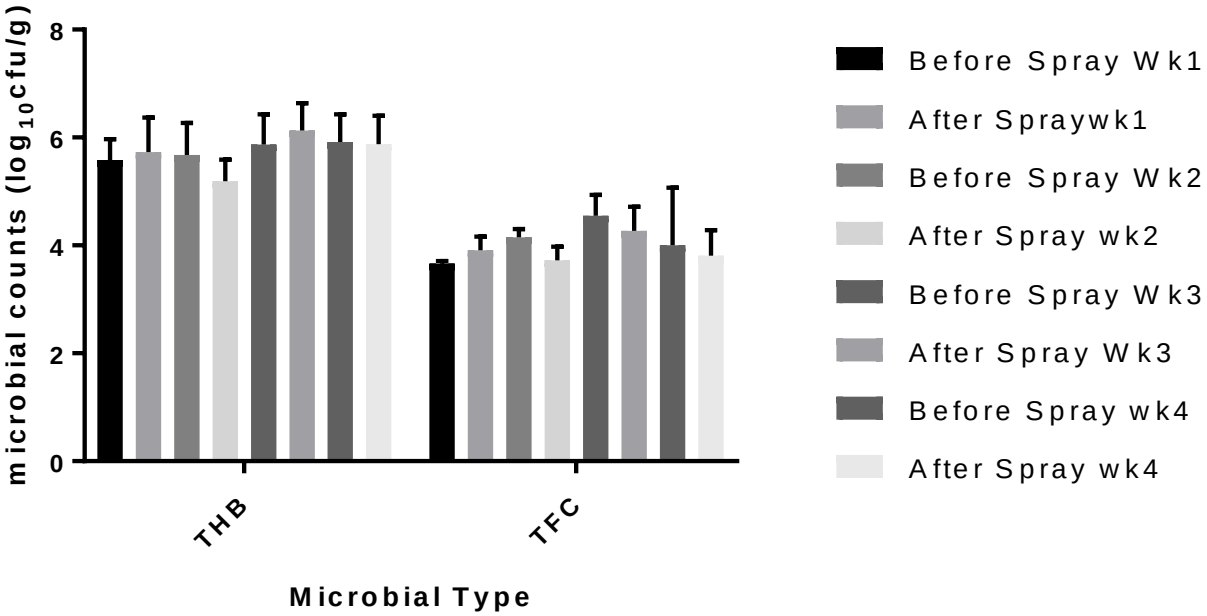

Fig 1: Bacterial and Fungal counts (Log_{10}cfu/g) before and after spray of pesticides in the farm.

The distribution of bacterial isolates identified in this study is presented in Table 1. The results showed uneven distribution of bacterial isolates. Total of seventy-four bacterial isolates belonging to: *Bacillus subtilis*, *Bacillus* sp, *Enterobacter* sp, *Micrococcus* sp, *Staphylococcus* sp, *P. aeruginosa*, *Bacillus cereus*, *Proteus* sp, *Bacillus mycoides*, *E. coli*, *B. licheniformis* and *Pseudomonas fluorescens* were identified. The results showed that some bacterial isolates such as *Bacillus subtilis*, *Micrococcus* sp, *Staphylococcus* sp, and *B. licheniformis* which were not isolated before the introduction of pesticide in the first week were isolated much later after introduction of pesticide while *Pseudomonas fluorescens* which was isolated before the introduction of pesticide was in subsequent weeks after pesticide introduction not isolated. Although some other bacterial isolates like *Bacillus mycoides* was very dominant as it was isolated throughout the study period irrespective of the week. The frequency occurrence of bacterial isolates presented in Figure 2 showed that *Bacillus mycoides* was the most predominant bacteria with frequency of 14.9% while *E. coli* was the second most predominant bacterial isolates having a frequency of 12.2%. The frequency occurrence of *Bacillus subtilis*, *Bacillus* sp, *Enterobacter* sp, *Micrococcus* sp, *Staphylococcus* sp, *P. aeruginosa*, *Bacillus cereus*, *Proteus* sp, *B. licheniformis* and *Pseudomonas fluorescens* were 10.8, 10.8, 4.1, 8.1, 5.4, 9.5, 9.5, 1.4, 14.9, 12.2, 8.1 and 5.4 %, respectively. Thus, *Proteus* sp was the least occurring bacterial isolates and was only isolated in week 4 after the introduction of pesticide. The presence of *E. coli* which is a faecal coliform, *Proteus* sp and *Enterobacter* could be attributed to the contamination of the farm with faecal matter from poultry droppings used to fertilize the farm.

The uneven distribution of bacterial isolates could be due to limiting nutrients, environmental factors as well as the presence or absence of the pesticide. Microorganisms do not live in isolation. Thus, since they are found living together with other microbes in the environment, interactions are bound to take place. These interactions could be positive like in mutualism or commensalism or it could result in a negative interaction like secretion of substances which

inhibit the growth of other organisms. Microbial succession in the soil environment may also be responsible for the observations made in this study.

Table 1: Distribution of Bacterial Isolates across the samples in the respective Weeks

Isolates	Before Spray				After Spray			
	Week1	Week2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
Bacillus subtilis	-	-	+	+	-	-	+	+
Bacillus sp	+	-	+	-	+	+	+	+
Enterobacter sp	+	+	-	+	+	-	-	+
Micrococcus sp	-	-	+	+	-	+	+	+
Staphylococcus sp	-	+	-	-	-	+	-	-
P. aeruginosa	+	-	+	-	-	+	-	+
Bacillus cereus	+	-	+	-	+	-	+	-
Proteus sp	+	-	-	-	-	-	-	+
Bacillus mycoides	+	+	+	+	+	+	+	+
E. coli	+	+	+	-	+	+	+	+
B. licheniformis	-	+	+	-	+	+	+	-
Pseudomonas fluorescens	+	-	-	-	-	-	-	-

Keys: + = isolated; - = not isolated

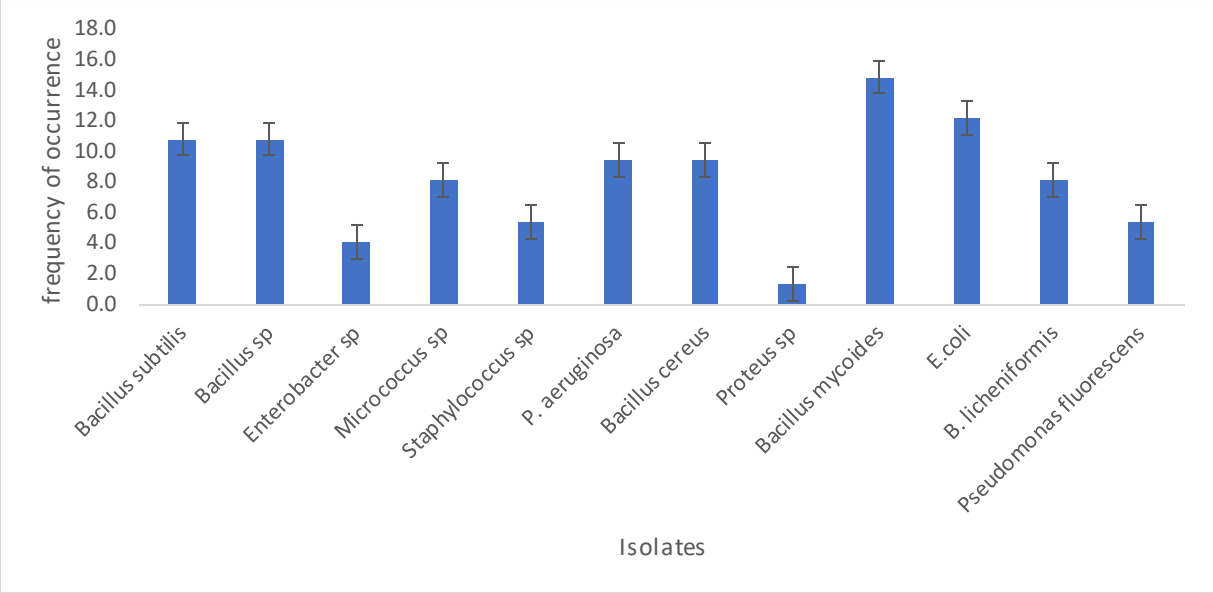

Figure 2: Frequency occurrence of Bacterial Isolates isolated from the soil

The results of the distribution of fungal isolates are presented in Table 2. Forty fungal isolates belonging to: *Penicillium* sp, *Saccharomyces* sp, *Mucor* sp, *Aspergillus flavus, Aspergillus niger, Microscporium* sp, *Aspergillus lentulus, Cladophialophora* sp, *Basidiobolus* sp, *Paceleomyces* sp, *Neosatorya* sp, *Rhizopus* sp and *Fusarium* sp were isolated from the various samples. Similar to the observation in the distribution of bacterial isolates across the samples in the different weeks, the fungal isolates were not also evenly distributed. The frequency occurrence of the fungal isolates is presented in Figure 3. The results showed that the frequency occurrence of *Penicillium* sp, *Saccharomyces* sp, *Mucor* sp, *Aspergillus flavus, Aspergillus niger, Microscporium* sp, *Aspergillus lentulus, Cladophialophora* sp,

Basidiobolus sp, *Paceleomyces* sp, *Neosatorya* sp, *Rhizopus* sp and *Fusarium* sp were: 15, 2.5, 7.5, 5, 20, 5, 2.5, 5, 5, 15, 2.5, 7.5 and 7.5 %, respectively. The most dominant fungal isolates were *A. niger* which was isolated in all the samples throughout the duration of study, while *Penicillium* sp and *Paceleomyces* sp were the second most dominant fungal isolates. *Saccharomyces* sp, *Aspergillus lentulus* and *Neosatorya* sp were the least frequent fungal isolates.

Table 2: Distribution of Fungal Isolates Across the samples in the Respective Weeks

Isolates	**Before Spray**				**After Spray**			
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Penicillium sp	-	-	+	+	+	+	+	+
Saccharomyces sp	-	-	-	-	+	-	-	-
Mucor sp	+	+	-	-	+	-	-	-
Aspergillus flavus	-	-	-	-	-	-	+	+
Aspergillus niger	+	+	+	+	+	+	+	+
Microscporium sp	+	-	-	-	+	-	-	-
Aspergillus lentulus	+	-	-	-	-	-	-	-
Cladophialophora sp	-	+	-	-	-	+	-	-
Basidiobolus sp	-	+	-	-	-	+	-	-
Paceleomyces sp	-	+	+	+	-	+	+	+
Neosatorya sp	-	+	-	-	-	-	-	-
Rhizopus sp	-	-	+	+	-	-	-	+
Fusarium sp	-	-	-	+	-	-	+	+

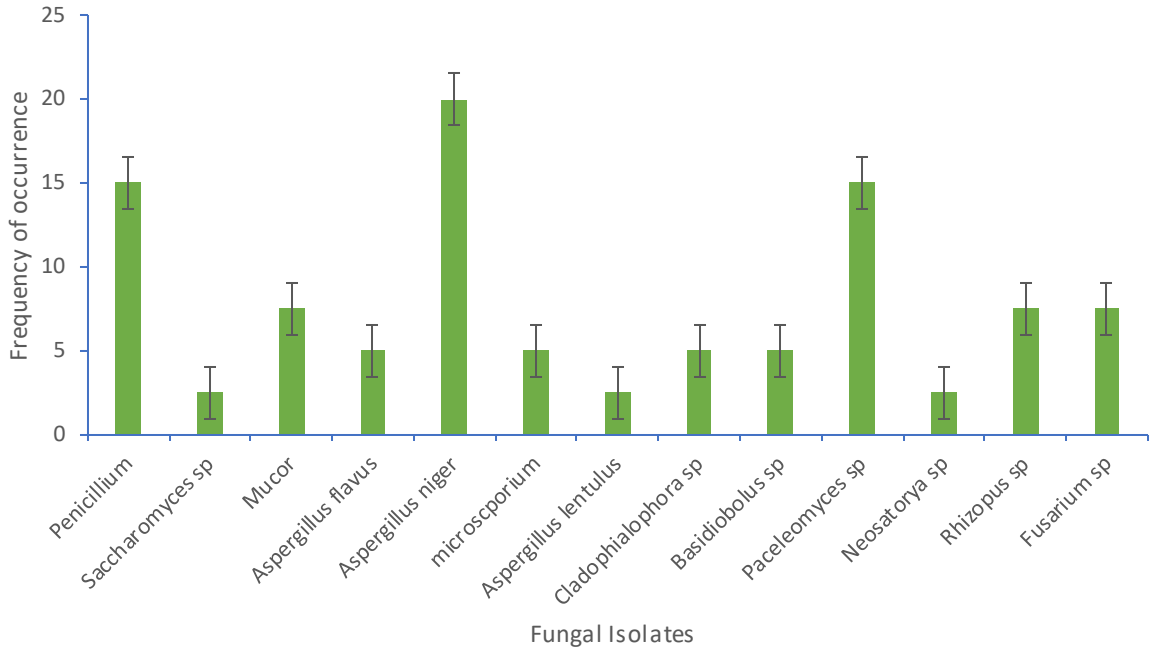

Fig. 3: Frequency occurrence of Bacterial Isolates isolated from the farm

Discussion

In other to reduce pest infestation on agricultural produce and crops, chemicals or substances are sprayed on farm produce or in the farms. Although the concentrations sprayed are not monitored as some local farmers are only concerned about eliminating crop pests and pay little or no attention on the detrimental effects these chemicals could pose in the environment and man who finally ingest this produce. According to the guidelines for the approval of pesticides, the effects of pesticides on soil microorganisms and soil fertility should be determined (Chi-Chulo, 2017). In this current study, the impact of pesticide on soil microorganisms showed that the pesticides affected the bacterial and fungal populations as well as their types. Research has shown that the continuous release of chemicals into the environment results in selective enrichment of microbial numbers, decrease in diversity and increase in the population of organisms that can utilize or withstand such chemicals (Douglas and Nwachukwu, 2016). However, it is worthy to note that the fertility of the soil is to a great extent dependent on the types and activities of microorganisms found in that soil. The findings in this current study in which the addition of pesticides decreased the bacterial and fungal populations agreed with the results of previous studies (Newman *et al.*, 2016; Aralujo *et al.*, 2003) who concluded that the presence of glyphosate decreased the number of bacteria, microbial biomass and acidobacteria population. Also, Mehjin *et al.* (2019) reported a decrease in bacterial population after treating soil samples with different pesticides. The decrease in bacterial population for a long time could slow down the pace at which some biogeochemical reactions are accomplished by these microorganisms (Newman *et al.*, 2016; Aralujo *et al.*, 2003). However, Partoazar *et al.* (2011) reported that the addition of pesticide increased the microbial growth and this agreed with the findings in this current study in which the bacterial populations increased in week 1 and 3, while fungal populations increased in week 1 after the addition of pesticide.

The species of *Bacillus* and *Pseudomonas* are among the bacterial isolates referred to as plant growth-promoting rhizobacteria (PGPR) which are free-living beneficial bacteria that offer health benefits to crop plants (Bashan *et al.*, 2014). This could explain the continuous dominance of the genera *Bacillus* in this current study. Kang *et al.* (2015a) identified

Pseudomonas and *Bacillus* spp as the predominant communities among the several species of plant growth-promoting bacteria (PGPB) and that due to their survival within diverse range of biotic and abiotic environments, they have been commercialized. The influence of the pesticides was also observed on the fungal diversity in the farm which resulted to uneven distribution of the fungal isolates. Research has shown that long term use of pesticides could result in the accumulation of these chemical residue in soil, affecting microbial population in the soil by favouring the growth of the organisms which are able to use them (Douglas and Dilosi, 2019).

The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides (Aurelia, 2009; Sethi *et al.,* 2013). This could be due to succession of fungal isolates. Some fungal isolates like *A. niger* were very dominant in the soil samples despite the presence of pesticide in the farm. While isolates such as *Mucor* sp, *Microscporium* sp and *Aspergillus lentulus* which were isolated before the introduction of pesticides in the first week were not isolated in the other weeks. This could mean that they lack the ability to utilize the pesticides as sources of energy and carbon source or they lack adaptive features that could withstand the effects of the pesticides. Although, other fungal isolates as presented in Table 2 were not isolated in the first week before and after introduction of pesticides but with continued introduction, they surfaced. Their presence could mean that the activities of other microorganisms especially those that were able to detoxify the pesticide have synthesized nutrients and made the environment favourable for their growth. In a previous study, it was demonstrated that microorganisms are capable to grow in the presence of several commercial pesticides and that activities such as catabolism and detoxification metabolism occur when soil microorganism uses the pesticide as carbon and energy source (Sanjay and Divya, 2016). Also, Douglas and Dilosi (2019) reported that certain microorganisms are able to tolerate certain pesticides by utilizing them as source of nutrient.

Conclusion

This study has demonstrated that the addition of chemical pesticide resulted in fluctuations in microbial populations and types. This could affect microbial activities such as nutrient recycling, decomposition, cause detrimental effects to the nutrient composition of the soil, affecting productivity and alter the ecological balance of the soil. Moreover, accumulation of chemical pesticides in the plants could also predispose consumers to consumption of chemical toxicants. Furthermore, laws guiding the use of pesticides as well as the concentrations to be used should be enforced. Routine monitoring of agricultural farms for pesticide pollution should be carried out.

Acknowledgement

The authors of this paper are grateful to Mrs Emelda Udochi Ebenezer for granting us access to her farm to collect samples and responding to all our questions asked.

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