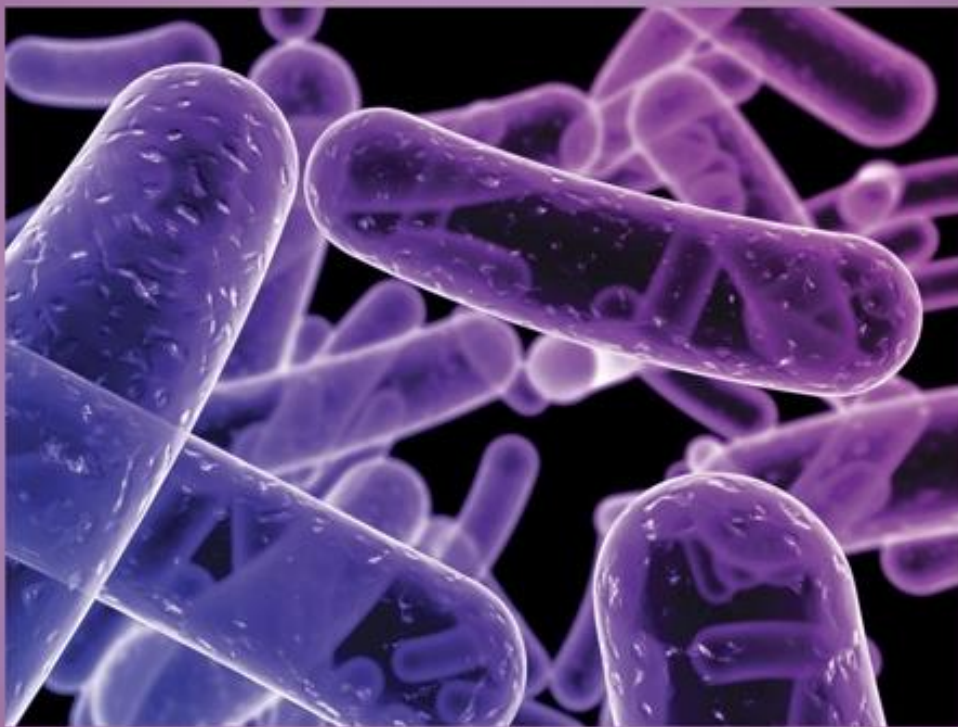




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Toxicity of Agricultural Pesticides to Soil Bacteria

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ABSTRACT

The annual application of pesticides to agricultural soil in a bid to increase crop yield is enormous. The consequence of this practice on bacterial population was assessed from soil collected from a control garden and two pesticide-contaminated vegetable farm soils. Eight pesticides; glyphosate, cypermethrin, 2,3-dichlorovinyl dimethyl phosphate (DDVP), paraquat, lambda-cyhalothrin, chlorpyrifos, emamectin benzoate and dichlorvos were applied to the soil at half the recommended field rate, recommended field rate (RFR) and double the recommended field rate. The control garden soil recorded the highest mean bacterial population compared to the pesticide-treated soil samples. Generally, glyphosate and lambda-cyhalothrin increased the bacteria population of the polluted farm soils with all the dose rates applied. Cypermethrin, DDVP and paraquat dramatically decreased the bacteria populations of the three soil samples. The 16S rRNA gene sequences showed that the bacteria belonged to the genera of *Bacillus*, *Thalassobacillus*, *Stenotrophomonas*, *Luteimonas* and *Pseudopropionibacterium*. Genes responsible for pesticide tolerance were not plasmid-mediated. The antibiotic susceptibility test showed that all the Gram-negative isolates were resistant to augmentin (30 µg), amoxicillin (2 µg) and sensitive to ofloxacin (5 µg), tetracycline (10 µg), nitrofurantoin (200 µg) and gentamicin (10 µg) while Gram-positive was resistant to ceftriaxone (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), cloxacillin (5 µg) and sensitive to ofloxacin (5 µg). This study showed that the application of agricultural pesticides affects the soil bacteria population and this can lead to a shift in soil health and fertility.

INTRODUCTION

Food insecurity is a global pandemic. In 'The State of Food Security and Nutrition in the World' 2020, it was estimated that almost 690 million (8.9 per cent) of the world's population went hungry in 2019, and the statistics keep rising (FAO, IFAD, UNICEF, WFP and WHO, 2020). One of the interventions aimed at stemming food insecurity is to increase crop yield. Healthy soils produce healthy crops and soil quality is directly linked to food quality and quantity (FAO, 2015).

In seeking ways to control disease carriers and different groups of pests, the agricultural industry witnessed a huge input of pesticides to maximize crop production and meet the demands for higher supplies of food for the fast-growing human population (Pal *et al.*, 2006).

Pesticide production started in India in 1952 (Aktar *et al.*, 2009) as a measure to increase crop yield. However, extensive use of pesticides affects the ecological and physical properties of soil. The populations and biological activity of soil microbes are impacted by pesticides thereby affecting their degradation capability (Zhou *et al.*, 2012). Indiscriminate use of pesticides does not just kill the targeted pests; it also affects non-target organisms and the environment including water, soil, air, and plants and it leads to the contamination of food (Glover-Amengor and Tetteh, 2008; Amaraneni, 2018). Also, farmers use pesticides on their intended targets without paying close attention to the recommended rate of application or the proper method for getting rid of any leftover pesticides. These present a problem for the soil's microorganisms, which has an impact on the health and fertility of the soil. The effects of pesticides on microbes may be direct or indirect and either beneficial or adverse (Staley *et al.*, 2015). Therefore, this study was aimed at determining the toxic effect of some commercially applied pesticides on soil bacterial survival and resistance to some available antibiotics.

MATERIALS AND METHODS

Soil Sampling:

The soil samples used were garden soil with no history of pesticide application (Sample A), soil from a vegetable farm (Sample B) in Mushin local government area of Lagos with a history of lindane (gamalin 20) application (latitude 6°31'7.60656" North and longitude 3°21'15.29748" East) and soil from agriculture farm extension Ikorodu, Lagos (Sample C), with history of chlorpyrifos (perfect killer) application

(latitude 6° 39'15.768" North and longitude 3°31'12.36" East). The soil samples were collected from different points on each farm at depths of 0-15 cm. The samples were thoroughly mixed to form a composite sample for each and then taken back to the laboratory for analysis. The soil samples were sieved to remove plant debris and stones before laboratory analysis.

Pesticides Used:

The commercially produced pesticides used were purchased from a local agricultural dealership store in Ojota, Lagos State, Nigeria (Table 1).

Soil Physicochemical Parameters:

The soil physicochemical parameters analyzed include soil moisture content (Zain *et al.*, 2013), pH (APHA, 1998), organic matter (APHA, 1998), particle size analysis; % silt, % clay and % sand (Bouyoucos, 1962), and % nitrogen and % phosphorus content (Jackson, 1973).

Soil Treatment and Pesticide Application:

Two hundred grams (200 g) of each soil sample were weighed into a sterile plastic container. The soil samples were pre-incubated in the dark at 25°C for 1 week for the adaptation of microorganisms before treatment with the pesticides. The soil was spiked and homogenized with the different doses of each pesticide. The soil treatment was carried out in three (3) different concentrations at half the recommended field rate, the recommended field rate and double the recommended field rate depending on the manufacturer's exposure period (days) in addition to the control sample (Table 1). The formula for calculating the treatments was applied as described by Adomako and Akyeampong (2016):

$$Y \left(\frac{\text{mg}}{\text{g}} \right) = \frac{\text{RFR (g a.i./ha)}}{\text{Am. AiF (g a.i./L)} \times 450 \text{ L/ha}} \times \frac{1000 \text{ mg}}{1 \text{ g}}$$

Where;

Y - Milligrams of chemical per gram of soil
RFR- Recommended Field Rate

Am. AiF - Amount of active ingredient in formulation
a.i. - active ingredient.

Table 1: Pesticide treatments per gram of soil

S/No	Common name	Active Ingredient	Class of pesticide	Type of pesticide	EC	mg a.i./g soil			Time of treatment
						RFR	Half RFR	Double RFR	
1	Perfect killer	Chlorpyrifos	Organophosphate	Insecticide	20%	138.89	69.44	277.78	7 days
2	Sniper	2,3-dichlorovinyl dimethylphosphate (DDVP)	Organophosphate	Insecticide	1000 g/l	0.0066	0.00333	0.0133	24 hours
3	Caterpillar force	Emamectin benzoate	Avermectin	Insecticide	5%	66.67	33.33	133.33	7 days
4	Ridoff	Dichlorvos	Organophosphate	Insecticide	100 g/l	0.044	0.022	0.088	7 days
5	Cypertex	Cypermethrin	Synthetic pyrethroid	Insecticide	100 g/l	2.22	1.11	4.44	4 days
6	Paraspring	Paraquat	Quaternary ammonium salts	Herbicide	20%	1.33	0.66	2.66	14 days
7	Attacke	Lambda-cyhalothrin	Pyrethroid	Insecticide	2.5%	5.33	2.66	10.66	7 days
8	Wuta-wuta	Glyphosate	Organophosphate	Herbicide	41%	135.5	67.7	271	7 days

RFR- Recommended Field Rate; a.i. – active ingredient; EC- Emulsifiable Concentrate

Bacteriological Analysis:

Bacterial Enumeration and Isolation:

The bacteria population in the soil was determined without any pesticide treatment to serve as the baseline to compare with the soils that were treated with the various pesticides. This was done for the three soil samples (A, B and C). The soil was collected from each microcosm 7 days after each treatment to assess the pesticides' effect on the bacterial populations in the soil. Nutrient agar (Oxoid) was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was done at ambient temperature ($28 \pm 2^\circ\text{C}$) for 24 – 48 h, bacterial colonies were counted and data were recorded. This was done in triplicate. Pure cultures of bacteria were isolated by the streak plate method (Adomako and Akyeampong, 2016).

Bacterial Identification:

Identification of bacteria was done as described by Yadav *et al.* (2015) and by molecular analysis.

Molecular Identification:

DNA Extraction:

The total genomic DNA extraction of the bacteria isolates was carried out according to the manufacturer's instruction using Bacterial DNA isolation kit SKU#10760111-1 (bio-World, Dublin Ohio). Pure cultures of fresh bacteria isolates were used for DNA extraction. The DNA was quantified in a spectrophotometer at A_{260} .

Polymerase Chain Reaction (PCR):

A 5 μl volume of the extracted DNA was amplified by PCR with Eppendorf Vapo protect thermal cycler (Nexus series). The Polymerase chain reaction (PCR) amplification of the 16S rRNA gene was carried out using the primer set 27F -5'-AGAGTTTGATCCTGGCT CAG-3', forward and 1492R -5'-GGTACCTTGTTACG ACTT-3', reverse (Aly *et al.*, 2017). The reaction was carried out in a 25 μl reaction mixture of Solis Biodyne 5x Hot FIREPol Blend master mix ready to load. The reaction was brought down to 1x concentration containing (1x blend master mix buffer, 1.5 mM Magnesium chloride (MgCl_2), 200 μM of each deoxynucleoside triphosphate (Solis Biodyne) and proofreading enzyme), 0.2 μM of each primer (10 pMol/ μl) and sterile double distilled water was used to make up the reaction mixture. The amplification condition was as follows: an initial denaturation step at 95°C for 15 mins, followed by 35 consecutive cycles of denaturation at 95°C for 30 secs, annealing at 61°C for 1 min 30 seconds and elongation time at 72°C for 1 min. After this, a final extension at 72°C for 10 mins was carried out. The PCR products were separated on a 1.5% agarose gel. Electrophoresis was done at 80 V for 1 hour 30 mins, and the DNA bands were viewed by ethidium bromide staining under UV transillumination and photographed by a gel documentation system. A 100 bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

DNA Sequencing:

All PCR products were purified and sent to Epoch Life science (USA) for Sanger sequencing. The sequences were edited by bioinformatics software Chromas. Analysis of the resulting DNA sequences was performed using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nih.gov/BLAST>) algorithm.

Plasmid Profiling:

The presence of plasmid DNA was confirmed by isolating plasmids from the pesticide-tolerating bacteria using the Fast-n-Easy plasmid mini-prep kit (Jena Bioscience, Germany). The isolated plasmid was characterized by agarose gel electrophoresis. The DNA band was observed under UV transillumination and photographed by a photo documentation system (Naphade *et al.*, 2012).

Antimicrobial Susceptibility Test:

Antibiotic susceptibility of the bacteria was determined using the agar disc diffusion method. The antibiotic disc used for the Gram-negative bacteria were; amoxicillin (2 µg), cotrimoxazole (25 µg), gentamicin (10 µg), nalidixic acid (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (200 µg) and tetracycline (10 µg). The antibiotic used against the Gram-positive bacteria were augmentin (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ceftriaxone (30 µg), ofloxacin (5 µg), cloxacillin (5 µg) and erythromycin (5 µg) (Abtek Biologicals Limited, United Kingdom). A fresh 24 h broth culture of each isolate adjusted to 0.5 McFarland standard (10^8 CFU/ml) was used to seed Muller-Hinton agar plates. The antimicrobial disc was placed on the Muller-Hilton agar plate after 15 mins of inoculation and each plate was incubated at

room temperature for 24 h. The plates were observed for zones of growth inhibition which were measured to the nearest millimeter with a ruler and data was recorded. The isolates were classified as resistant (R), intermediate (I), and sensitive (S) according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). Multiple Antibiotic Resistance (MAR) index and % MAR were calculated for each isolate (Krumperman, 1983).

Statistical Analysis:

The mean values and standard deviation were calculated. Data generated from bacterial populations were computed and subsequently expressed in tables and graphs using MS Excel 19. The effects of the agricultural pesticides on the bacteria population were also evaluated. Statistical significance ($P < 0.05$) of differences was analyzed by one-way analysis of variance (ANOVA) to compare the mean values between the baseline determinations and pesticide treatments and to bring out the differences that exist between the treated soils using STATA software. Differences were considered to be significant at $P < 0.05$.

RESULTS**Physicochemical Parameters of Soil:**

The physicochemical parameters of the soil samples in Table 2 show that the pH and moisture contents of the soil were within acceptable limits. Sample C had the highest available nitrogen (0.17) followed by sample A (0.14), then sample B (0.09). Sample A had the highest % carbon followed by B and C. Sample B showed the highest % phosphorus (0.020) followed by samples A and C. The soil samples were classified as loamy sand soil (A) and sandy loam soils (samples B and C) based on the particle size distribution of percentage silt, sand and clay (Lotus Arise, 2021).

Table 2: Physiochemical parameters of the soil

Parameters	Soil A	Soil B	Soil C
pH	7.10	3.40	5.90
Moisture content (%)	10.40	14.46	9.41
Carbon (%)	10.60	10.10	6.60
Nitrogen (%)	0.14	0.09	0.17
Phosphorus (%)	0.012	0.020	0.005
Silt (%)	7.00	14.00	12.00
Clay (%)	7.00	10.00	18.00
Sand (%)	85.00	75.00	63.00
Gravel (%)	1.00	1.00	7.00
Particle size distribution	Loamy sand	Sandy loam	Sandy loam

A, Garden soil; B, Pesticide contaminated vegetable soil; C, Pesticide contaminated vegetable soil

Effect of Agricultural Pesticides on Soil Bacteria Isolated from Soil Samples A, B and C:

The mean bacterial population of soil samples treated with eight different pesticides at the manufacturer's recommended field rate (RFR), less RFR and double RFR is illustrated in Figures 1 - 6. The Figures 1-3 depict the effect of pesticide application on the bacteria population in the soil, while Figure 4-6 shows the effect of the different pesticide treatments on soil bacteria.

There was a decrease in the bacteria population at all doses applied compared to the control ($> 10^9$ CFU/g of soil) in the unpolluted garden soil without pesticide application (Fig. 1). The bacterial population in the unpolluted garden soil was more affected by Emamectin benzoate, cypermethrin and DDVP applications, with a mean population of 10^7 CFU/g of soil. At the recommended field rate, the population of bacteria was relatively unaffected in lambda-cyhalothrin, dichlorvos, chlorpyrifos and paraquat ($>10^8$ CFU/g of soil, Figure 1). The result of the effect of different treatment rates of pesticide application on soils from

farmlands with a history of pesticide application in Figures 2-3, showed that overall, pesticide application had a positive impact on the bacteria populations. The bacteria population in the farmlands (Figs. 2-3) was comparatively lower (10^8 CFU/g) than the population from the unpolluted garden soil in Figure 1 (10^9 CFU/g). Generally, glyphosate and lambda-cyhalothrin increased the bacteria population with all the dose rates applied. DDVP impacted the soil bacterial population the most negatively. Applying a one-way analysis of variance on the toxicity of the pesticide to the soil bacteria, there was no significant difference ($P < 0.05$) in the soil treatments for each pesticide (RFR, Less RFR, Double RFR) for the individual soil samples (Table 3). The control soil without pesticide application in Figures 1 and 4-6, had more bacteria population ($> 1.4 \times 10^9$ CFU/g of soil) than the pesticide applied soils, followed by the recommended field rate (RFR) ($< 1.2 \times 10^9$ CFU/g of soil). The bacterial population was more negatively impacted by double RFR than the less RFR (Figs. 5-6).

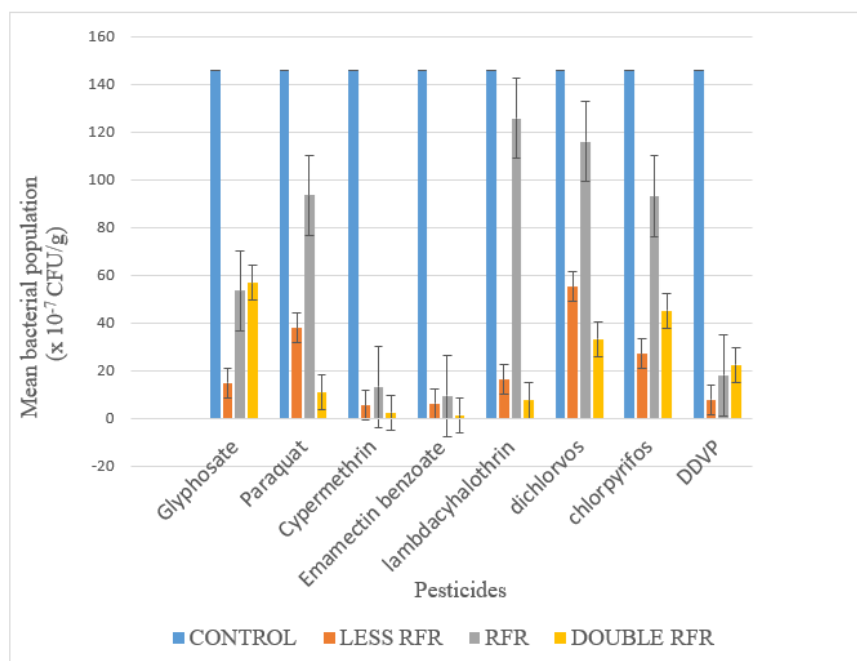


Fig. 1: Effect of agricultural pesticides on soil bacteria isolated from unpolluted garden soil A. RFR-Recommended field rate.

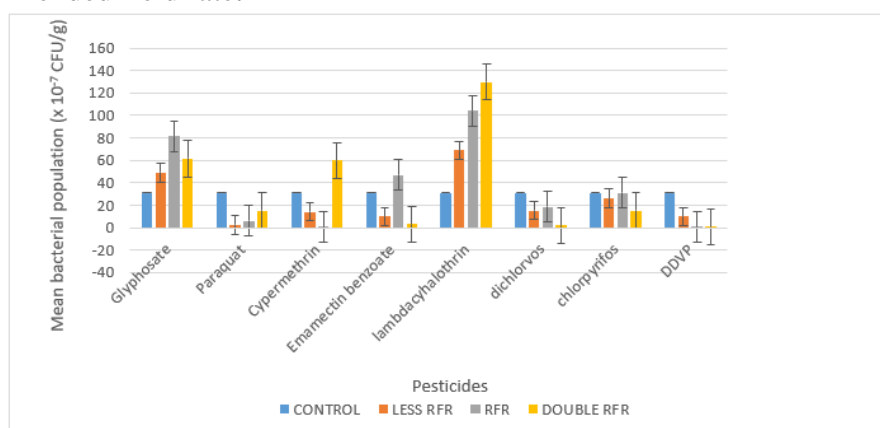


Fig. 2: Effect of agricultural pesticides on soil bacteria isolated from polluted farm soil B. RFR-Recommended field rate.

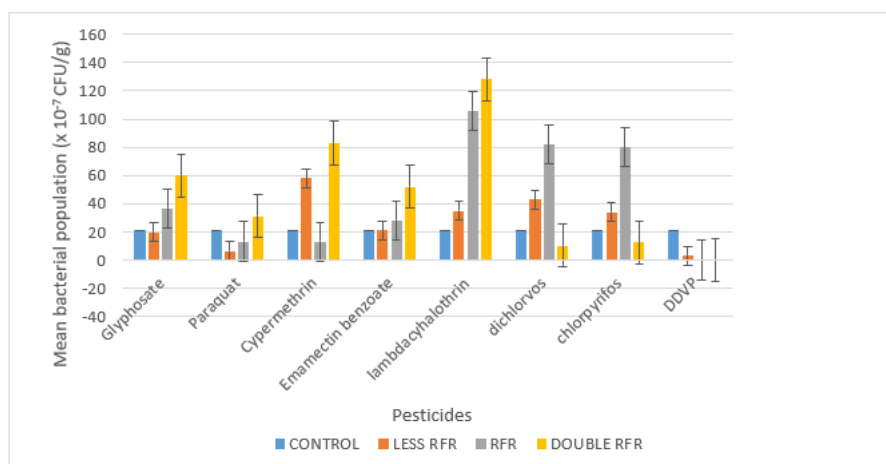


Fig. 3: Effect of agricultural pesticides on soil bacteria isolated from polluted farm soil C. RFR-Recommended field rate.

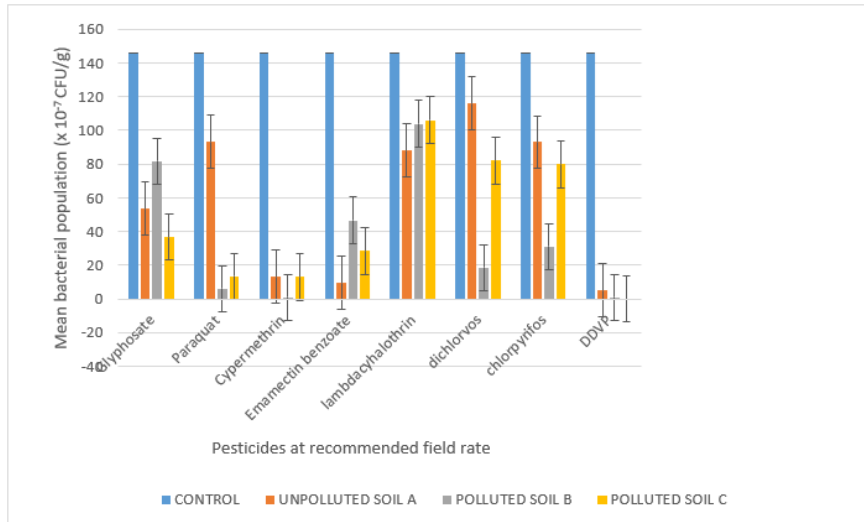


Fig. 4: Effect of pesticide application at the recommended field rate on soil bacterial population.

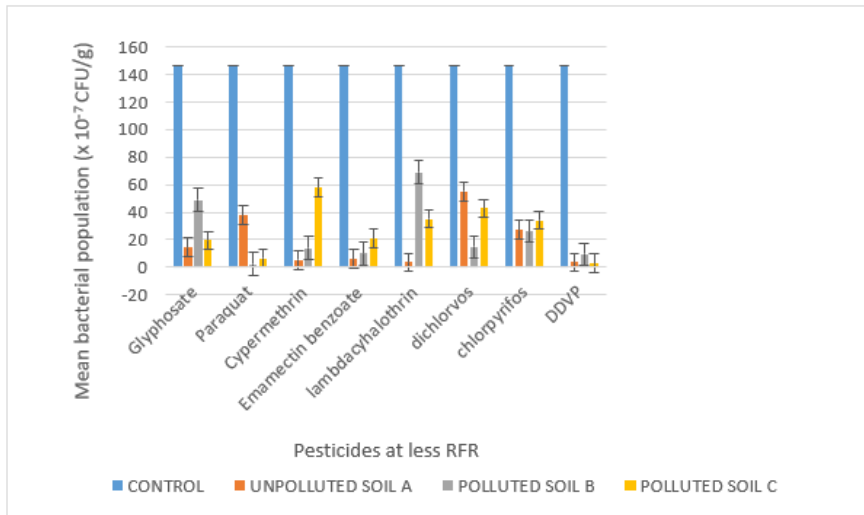


Fig. 5: Effect of pesticide application at less than the recommended field rate on soil bacterial population.

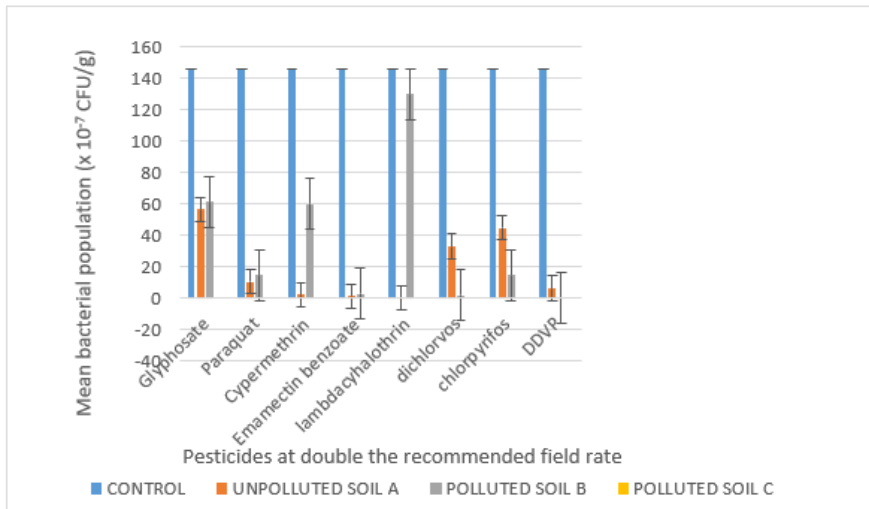


Fig. 6: Effect of pesticide application at double the recommended field rate on soil bacterial population.

Table 3: One-way analysis of variance of the effect of pesticide treatment on Soil samples.

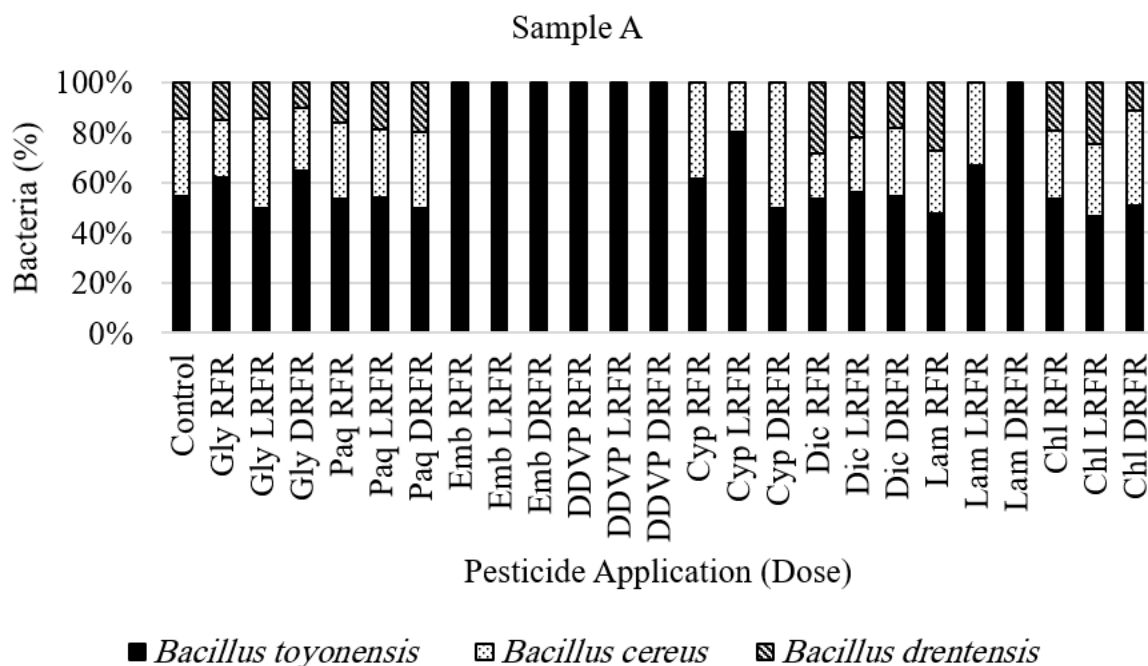
Soil Sample	Pesticide Treatment (mg a.i./g soil) *		
	RFR	LESS RFR	DOUBLE RFR
Unpolluted Garden Soil A	P > F =0.8845	P > F =0.891	P > F =0.9841
Farmland Soil B	P > F =0.9334	P > F =0.9929	P > F =0.9986
Farmland Soil C	P > F =0.9804	P > F =0.9967	P > F =0.9997

* Significant Difference ($P < 0.05$); P, Probability; F, Frequency.

Frequency of Distribution of Bacteria in Soil:

The distribution of the bacteria isolates in the treated soil samples is represented in Figures 7-9. *Bacillus toyonensis*, *B. cereus* and *B. drentensis* were prevalent in the unpolluted garden soil after-treatment of the soil (Fig. 7). *Bacillus toyonensis* predominated in the soil after treatment with all the pesticides and was the only organism present in the soil treated with emamectin and DDVP. The bacteria present in the soil Sample B include *Stenotrophomonas pavanii*, *Thalassobacillus*

cyri and *Enterobacter* sp. *Stenotrophomonas pavanii* and *Enterobacter* sp. were the most prevalent in the soil with each as the only isolate present in Sample B soil treated with cypermethrin and DDVP respectively (Fig.8). The bacteria, *Stenotrophomonas pavanii*, *Stenotrophomonas maltophilia*, *Luteimonas arsenica*, *Pseudopropionibacterium propionicum* and *Aeromonas* sp. were present and evenly distributed in farmland soil sample C (Fig. 9). However, the soil treated with DDVP had only *Luteimonas arsenica* present in the treatment with Less than the RFR.

**Fig. 7:** Percentage distribution of bacteria in unpolluted garden soil (Sample A).

GLy, Glyphosate; Paq, Paraquat; Emb, Emamectin benzoate; DDVP, 2,3-dichlorovinyl dimethyl phosphate; Cyp, Cypermethrin; Dic, Dichlorvos; Lam, Lambda-cyhalothrin; Chl, Chlorpyrifos.

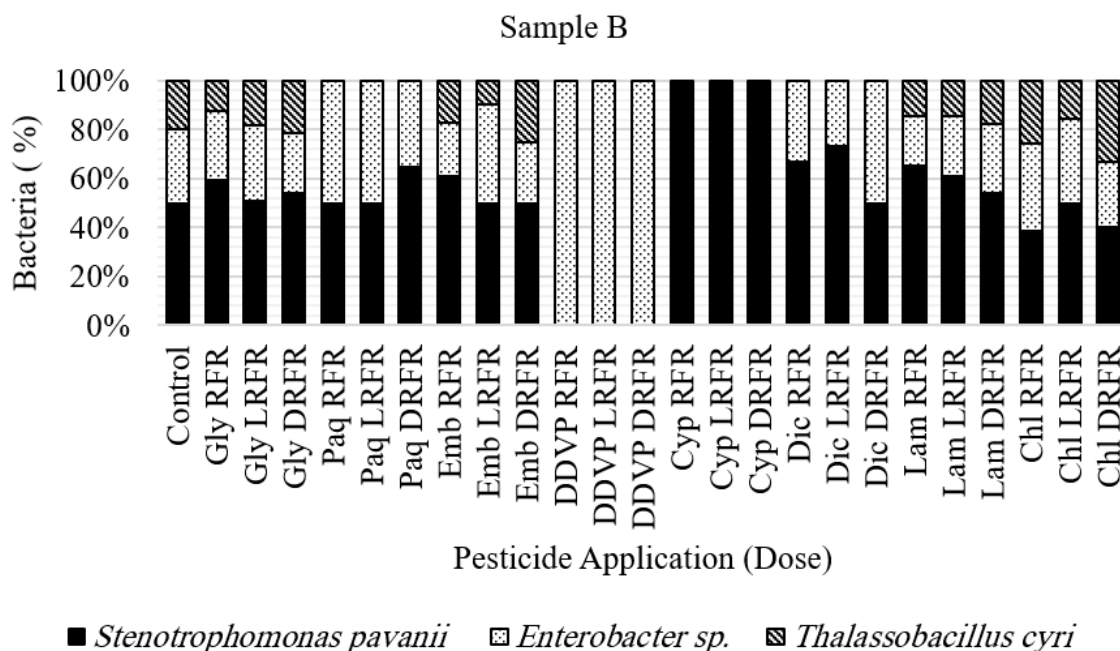


Fig. 8: Percentage distribution of bacteria in polluted farmland soil (Sample B). GLy, Glyphosate; Paq, Paraquat; Emb, Emamectin benzoate; DDVP, 2,3-dichlorovinyl dimethyl phosphate; Cyp, Cypermethrin; Dic, Dichlorvos; Lam, Lambda-cyhalothrin; Chl, Chlorpyrifos.

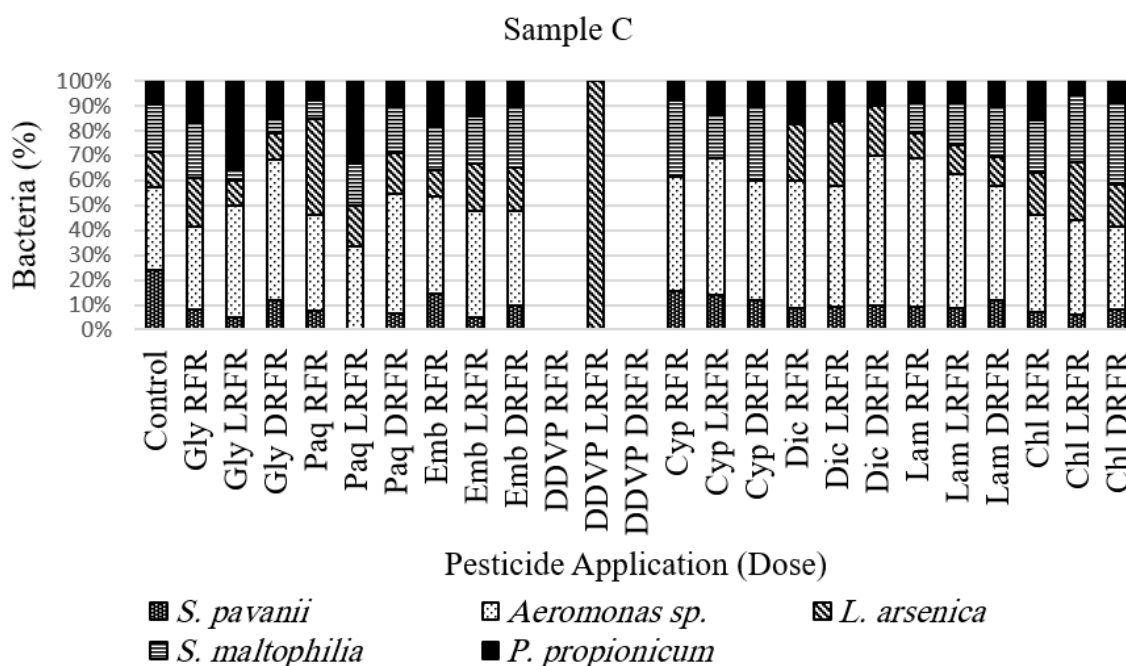


Fig. 9: Percentage distribution of bacteria in polluted farmland soil (Sample C). GLy, Glyphosate; Paq, Paraquat; Emb, Emamectin benzoate; DDVP, 2,3-dichlorovinyl dimethyl phosphate; Cyp, Cypermethrin; Dic, Dichlorvos; Lam, Lambda-cyhalothrin; Chl, Chlorpyrifos; P, *Pseudopropionibacterium*; S, *Stenotrophomonas*; L, *Luteimonas*.

Antibiotic Susceptibility:

The bacteria isolates exhibited multiple resistance to 3 or more antibiotics in

the susceptibility test in Tables 4 and 5. The Gram-negative bacteria were all resistant to amoxicillin (2 µg), cotrimoxazole (25 µg) and

augmentin (30 µg); while they were all susceptible to ofloxacin (5 µg). Their % MAR Index was between 38 - 89% (Table 4). All the Gram-positive bacteria were resistant to ceftazidime (30 µg), cefuroxime (30 µg),

ceftriaxone (30 µg), cloxacillin (5 µg) and augmentin (30 µg). They were all sensitive to gentamicin (10 µg), erythromycin (5 µg) and ofloxacin (5 µg). And had a MAR index of 63%. None of the bacteria carried plasmids.

Table 4: Antibiotic susceptibility of Gram-negative bacteria from pesticide polluted soils.

Isolate	plasmid	Antibiotics								MAR Index (%)
		AMX (2 µg)	COT (25 µg)	NIT (200 µg)	GEN (10 µg)	NAL (5 µg)	OFL (5 µg)	AUG (30 µg)	TET (10 µg)	
<i>Luteimonas arsenica</i>	-	0(R)	0(R)	23(S)	20(S)	20(S)	21(S)	0(R)	20(S)	38%
<i>Stenotrophomonas pavanii A1</i>	-	0(R)	0(R)	14(S)	15.5(S)	20(S)	25(S)	0(R)	12(S)	38%
<i>Stenotrophomonas pavanii A2</i>	-	0(R)	0(R)	15(S)	14(S)	20(S)	26(S)	0(R)	12(S)	38%
<i>Stenotrophomonas maltophilia</i>	-	0(R)	0(R)	15(S)	12(S)	21(S)	22(S)	0(R)	10(S)	38%
<i>Enterobacter species</i>	-	0(R)	0(R)	0(R)	0(R)	23.5(S)	25(S)	0(R)	12(S)	63%
<i>Aeromonas species</i>	-	0(R)	0(R)	0(R)	0(R)	0(R)	10(S)	0(R)	0(R)	89%

AMX, Amoxicillin; COT, Cotrimoxazole; NIT, Nitrofurantoin; GEN, Gentamicin; NAL, Nalidixic acid; OFL, Ofloxacin; AUG, Augmentin; TET, Tetracycline; S, Sensitive; R, Resistant; MAR, Multiple Antibiotic Resistance.

Table 5: Antibiotic susceptibility of Gram-positive bacteria from pesticide-polluted soils.

Isolate	plasmid	Antibiotics								MAR Index (%)
		CAZ (30 µg)	CRX (30 µg)	GEN (10 µg)	CTR (30 µg)	ERY (5 µg)	CXC (5 µg)	OFL (5 µg)	AUG (30 µg)	
<i>Bacillus toyonensis</i>	-	0(R)	0(R)	18(S)	0(R)	16(S)	0(R)	24(S)	0(R)	63%
<i>Bacillus cereus</i>	-	0(R)	0(R)	14(S)	0(R)	15(S)	0(R)	20(S)	0(R)	63%
<i>Pseudopropionibacterium propionicum</i>	-	0(R)	0(R)	13(S)	0(R)	20(S)	0(R)	25(S)	0(R)	63%
<i>Thalassobacillus cyri</i>	-	0(R)	0(R)	16(S)	0(R)	14(S)	0(R)	24(S)	0(R)	63%
<i>Bacillus drentensis</i>	-	0(R)	0(R)	21(S)	0(R)	14.5(S)	0(R)	32(S)	0(R)	63%

AUG, Augmentin; CAZ, Ceftazidime; CRX, Cefuroxime; GEN, Gentamicin; CTR, Ceftriaxone; OFL, Ofloxacin; CXC, Cloxacillin; ERY, Erythromycin; S, Sensitive; R, Resistant; MAR, Multiple Antibiotic Resistance.

DISCUSSION

This study showed the effect of pesticides on soil bacterial growth and population. Based on the particle size distribution of percentage silt, sand and clay, the soil textures were classified as loamy sand soil (A) and sandy loam soils (samples B and C) (USDA-NRCS, 1999; Lotus Arise, 2021). Soil texture affects the nutrient supply of the soil (Gupta and Shukla, 1991) and variability in soil texture can directly or indirectly influence many soil functions and soil threats such as soil erosion (Adhikari *et al.*, 2009; Tale and Ingole, 2015). Generally, loamy soils are 'all round' soils and may be used to grow most crops (Abubakar, 2017). The pH

of the soil samples showed variations between 3.4 to 7.1. Soil pH is one of the most important physicochemical parameters of soil. It affects mineral nutrients, soil quality and microbial activity (Chaudhari, 2013). According to Fernández-Calviño and Bååth (2010), bacterial growth is highly influenced by pH, showing optimum growth at a pH related to the soil pH. Soil moisture content is another important factor affecting the soil environment. A high moisture content limits the decomposition of organic matter due to low oxygen supply, while low soil moisture decreases microbial activity by reducing the diffusion of soluble substrates, microbial mobility and intracellular water potential

(Stark and Firestone, 1995; Schjøning *et al.*, 2003; Sierra *et al.*, 2017). Soil water content may also determine microbial community structure (Stres *et al.*, 2008). Soil organic carbon is the basis of soil fertility. Soil organic carbon tends to be concentrated in the topsoil. It ranges from 0.5% to 3.0% for most upland (Athira *et al.*, 2019). Soils containing at least 12–18% organic carbon are generally classified as organic soils (Allen, 2012). The soil from this study falls within the topsoil and organic soil range. Percentage concentrations of N and P are also in the normal range (Chaudhari, 2013). Nitrogen and phosphorus are important nutrients for growth and development in microorganisms.

Pesticide treatments of soils at different application rates with glyphosate, emamectin benzoate, DDVP, paraquat, cypermethrin, chlorpyrifos, lambda-cyhalothrin and dichlorvos showed significant effects on microbial growth. The three levels of pesticide concentrations (manufacturer's recommended field rate (RFR), less RFR and double RFR) had both positive (stimulatory) and negative (inhibitory) impacts on the growth of microorganisms. According to Lo (2010). Some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms. It was observed that the viable count of bacteria was highest in the control soil from the unpolluted garden soil than in soils treated with the different pesticides. This showed that application of the pesticides decreased the bacteria population and hence microbial activity in the soil. This observation is consistent with the findings of Sebiomo *et al.* (2011) and Santhoshkumar *et al.* (2015), that soil bacterial populations in control samples were noticeably greater than those in soils treated with herbicides.

Generally, glyphosate and lambda-cyhalothrin increased the bacteria population with all the dose rates applied, while cypermethrin, paraquat and DDVP decreased the population. According to Partoazar *et al.* (2011) and Haney *et al.* (2000), soil with the highest long-term exposure to herbicides

exhibits the strongest relationship to microbial activity. Bacterial growth in the presence of glyphosate as the sole C source indicates that the glyphosate was a source of energy for microbial activity. Several studies have reported an increase in microbial population during the application of glyphosate to soil (Partoazar *et al.*, 2011; Lane *et al.* (2012); Arora and Sahni (2016); Haney *et al.*, 2000).

The study by Naré *et al.* (2019) demonstrated the contribution of microbial activity and concomitant degradation of lambda-cyhalothrin in soil. Latif *et al.* (2008), reported an enhanced soil microbial population immediately after the application of flubendiamide, nimbicidine, lambda-cyhalothrin, abamectin and thiodicarb pesticides and a retained stimulatory effect even after 32 days of application. Similarly, on days 14 and 28, Cyco *et al.* (2006) found a greater rise in bacteria number in lambda-cyhalothrin-treated soils compared to the control. It's possible that the pesticides' stimulatory effects result from microbes mineralizing soil organic matter and utilizing some of the formulations' less-pure chemical components as nutrition.

A decrease in soil bacteria population was observed when cypermethrin was applied to unpolluted garden soil at different field rates. The reduction in the bacteria population on the application of cypermethrin to soil was similarly observed by Goswami *et al.* (2013) and Adebisi *et al.* (2018). According to Adebisi *et al.* (2018), there were significant reductions in bacterial populations in the first 14 days at both normal field concentration (NFC) and X5FC. However, Gundi *et al.* (2005) demonstrated that even at the highest concentration of $25 \mu\text{g g}^{-1}$, the insecticides monocrotophos, quinalphos, and cypermethrin considerably increased the growth of bacteria and fungi as well as the soil dehydrogenase activity. However, when the two insecticides (monocrotophos or quinalphos + cypermethrin) were present together in the soil at the highest level ($25 + 25 \mu\text{g g}^{-1}$), antagonistic interactions were more pronounced toward soil microflora and

dehydrogenase activity, whereas synergistic or additive responses occurred at lower levels with the same combination of insecticides in soil. Jilani and Khan (2006), observed that at a high concentration of cypermethrin, the number of organisms decreased or very slightly increased. These observations by Jilani and Khan (2006) and Gundi *et al.* (2005) summarize the effect of the pesticides observed in the soil sample treatment in this study.

Application of chlorpyrifos treatment to soil resulted in a reduced bacterial population. Similar results were observed by other researchers (Ahmed and Ahmed, 2006; Santhoshkumar *et al.* 2015; Arora and Sahni, 2016). Ahmed and Ahmed (2006) reported that the application of insecticides chlorpyrifos, imidacloprid, cypermethrin, endosulfan and carbofuran under field conditions causes considerable variation in soil bacterial populations and a significant reduction in bacteria population and diversity in laboratory conditions. Among the applied insecticides, chlorpyrifos had the most destructive effect on soil bacterial diversity. In a study by Hussien *et al.* (2012) on the effect of chlorpyrifos, lambda-cyhalothrin and emamectin benzoate at field application rate on the total population count of microorganisms in *Phaseolus vulgaris* field, they observed that chlorpyrifos stimulated significantly the proliferation of all of the microorganisms. Lambda-cyhalothrin decreased the population of Bacteria at 1 DAT (the day after treatment) until 15 DAT and returned to increase at 21 DAT. Emamectin benzoate in general increased the population of bacteria and actinomycetes and decreased the population of fungi (Hussien *et al.*, 2012). However, the data on the effect of emamectin benzoate in this study showed that the pesticide had a negative effect on the bacterial population in the soil samples. Cenkseven *et al.* (2019) made a similar observation on soil microbial activity while investigating the effects of emamectin benzoate at different recommended field doses (RFD, RFD×2, RFD×4) on soil carbon mineralisation.

Zain *et al.* (2013) reported a drastic inhibition of both bacterial and actinomycetes populations by Paraquat at less RFR and to about 70 to 82% at the recommended rate. Similarly, Adomako and Akyeampong, (2016) reported that Paraquat treatment recorded an increase by 87.2% of the bacterial population for the first 5DAT but the effect of its toxicity was felt from 10DAT (6.5%) to the 15DAT (6.4%). The inhibitory capacity of Paraquat stems from the fact that it is known to be bounded strongly and coherently to soil components, including clay minerals and organic matter, therefore limiting the access of microorganisms to Paraquat in soil water (Bromilow, 2004). This characteristic conversely minimizes the possible adverse influence of paraquat on soil-dwelling organisms (Bromilow, 2004). The result of this study presents a one-off sampling after the application of the herbicide and the decrease in bacterial population was also observed by Devashree *et al.* (2014) in the bacterial population of the rhizosphere microflora of tea and non-rhizosphere soil, where microorganisms were significantly affected at the initial stages of paraquat application.

None of the eight tested pesticides had a consistently negative effect on bacterial activity except DDVP. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate), commonly abbreviated as DDVP, was reported to have a toxic effect on microorganisms as a general decline in microbial population post-application of the pesticide at recommended rates in soil was demonstrated by Bankole *et al.* (2020). Also, Adigun *et al.* (2022), reported a decline in soil microflora after the application of dichlorvos to two soil types (clay and sand) over the course of three weeks of application. Just as observed by Adebisi *et al.* (2018), the results of the effect of pesticide treatment indicated that pesticide concentration affected microbial numbers and was dependent on the pesticide type.

The genera of bacteria isolated have been shown to be versatile degraders of pesticides (Tu and Bollen, 2006; Dubey and

Fulekar, 2013; Ishag *et al.*, 2016; Bhatt *et al.*, 2019). The strains were identified as *Aeromonas* sp., *Bacillus toyonensis*, *Bacillus cereus*, *Bacillus drentensis*, *Enterococcus* sp., *Stenotrophomonas pavanii*, *Thalassobacillus cyri*, *Stenotrophomonas pavanii*, *Luteimonas arsenica*, *Stenotrophomonas maltophilia* and *Pseudopropionibacterium propionicum*. *Bacillus* species are well known as highly resistant spore-forming bacteria. They possess excellent characteristics and are extremely efficient for many agricultural, environmental and industrial applications (El-Bestawy *et al.*, 2013). *Bacillus* species have been reported to be excellent degraders of cypermethrin (Bhatt *et al.*, 2019), DDVP (Zhang *et al.*, 2021) and glyphosate (Aso *et al.*, 2021). *Bacillus cereus* strains have been reported to degrade various pesticides like chlorpyrifos, Malathion (Ishag *et al.*, 2016) and paraquat (Tu and Bollen, 2006). *Bacillus cereus*, *Stenotrophomonas maltophilia* and *Enterobacter aerogenes* indicated a high capacity to be able to degrade glyphosate (Aso *et al.*, 2021). *Stenotrophomonas maltophilia* was reported to degrade cypermethrin (Dubey and Fulekar, 2013) and lambda-cyhalothrin (Onuorah *et al.*, 2020) in soil. Several bacteria that are capable of degrading dichlorvos have been reported; these include *Flavobacterium* sp., *Pseudomonas* spp., *Proteus vulgaris*, *Vibrio* spp., *Serratia* spp. and *Acinetobacter* spp. (Ning *et al.*, 2012; Agarry *et al.*, 2013; Parte *et al.*, 2020). Several bacteria capable of utilizing paraquat as the sole growth source of carbon and nitrogen were reported by Tu and Bollen (2006). These bacteria include, *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Pseudomonas fluorescens*, and *Bacillus cereus* (Tu and Bollen, 2006). Emamectin benzoate was shown to be degraded by *Achromobacter* spp. and *Diaphorobacter* sp. (Rahman *et al.*, 2018).

Pesticide degradative genes in microbes have been found to be located on plasmids, transposons, and/or chromosomes (Kumar *et al.*, 1996). Plasmid profiling of the bacteria isolates from the soil samples showed the absence of plasmid. This indicated that no

plasmids were involved in pesticide tolerance and the genes responsible for the pesticides may be located on the bacterial chromosome. Similarly, Ibrahim *et al.* (2015), indicated that no plasmids were involved in pesticide degradation, and genes responsible for Malathion, granstar and topik degradation were located on the bacterial chromosome.

The antibiotic profiling showed that the bacteria had multiple resistance to 3 or more antibiotics. Assessment of the antibiotic resistance among the bacteria isolated from the pesticide-contaminated soil is important for the update on bacterial antibiotic resistance patterns. There have been reports on the detection of antibiotic resistance genes in bacteria isolated from agricultural soil that can be transferred to humans through the food chain (Founou *et al.*, 2016; Manyi-Loh *et al.*, 2018; Zalewska *et al.*, 2021). Li *et al.* (2019), isolated *S. maltophilia* from multiple hydroponically produced leafy green vegetables (sweet basil, kale, and parsley) and from the nutrient solution used by a hydroponic farm. *Stenotrophomonas maltophilia* is an emerging opportunistic pathogen that causes severe nosocomial infection in immunocompromised populations with a high mortality rate (Li *et al.*, 2019). Lucena-Adrós *et al.* (2014), isolated *Propionibacterium olivae* sp. nov. and *Propionibacterium damnosum* sp. nov. from spoiled packaged Spanish-style green olives; and there are several reports on the isolation of *Bacillus cereus* from vegetables (Valero *et al.*, 2002; Gdoura-Ben Amor *et al.*, 2018; Yu *et al.*, 2019). The isolation of some of the bacteria in this study with multiple resistance to antibiotics in food from other studies (Valero *et al.*, 2002; Lucena-Adrós *et al.*, 2014; Gdoura-Ben Amor *et al.*, 2018; Li *et al.*, 2019; Yu *et al.*, 2019) is of concern since this can lead to the transfer and development of antibiotic resistance in humans and animals.

It is crucial to realize that soil microorganisms may become more resistant to many drugs as a result of pesticide presence and persistence in agricultural soil. Therefore, it's important to comprehend how pesticides

affect soil microbial populations before approving them for use in agriculture and industry. Additionally, limiting the negative environmental effects of pesticide application requires educating farmers and end-users on pesticide use.

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