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Tolerance of *Pseudomonas* and *Bacillus* spp. to Cyahalothrin and Chlorpyrifos Pesticides

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ABSTRACT

The aim of this research is to determine the tolerance of *Pseudomonas species* and *Bacillus species* to Chloropyrifos and Cyahalothrin pesticides. The study area was the Rivers State University school farm, Faculty of Agriculture, Rivers State, Nigeria. The University farm is a large area of land that specializes in fish farming, livestock farming, poultry farming and all types of agricultural product farming. The university farm has also been used by students for research purposes. Standard microbiological procedures were used; Nutrient agar was prepared by weighing 28g of nutrient agar into 1000ml of distilled water in Erlenmeyer flask. The medium was sterilized at 121ºC for 15 minutes using the autoclave at 15psi. centrimide agar was prepared by weighing 45.3g of the agar and measuring 10ml of glycerol in 990ml of distilled water. The medium was heated with frequent agitation and boiled to completely dissolve the medium before autoclaving at 121ºC for 15 minutes. Toxicity testing procedures were carried out by preparing a stock culture of the pesticide based on directions (8ml into 1000ml of distilled water) from which the concentrations used for this research was obtained 0%, 3.125%, 6.25%, 12.5%, 25% and 50% and tested on the soil sample for a period of 28 days. Samples were serially diluted and cultures were incubated at 35^0C for 18 to 24 hours. LC_{50} was determined using SPSS version 2.0. Acute toxicity analysis was carried on pesticides (Chlorpyrifos and Cyahalothrin) in soil using *Bacillus* and *Pseudomonas* species as bio indicators. The toxicity results obtained in this study revealed that the pesticides (Chloropyrifos and Cyahalothrin) were toxic to the microorganisms. The results of median lethal concentration (LC_{50}) of the pesticides to the bio indicators (*Pseudomonas* and *Bacillus* species) which were determined by subtracting the value of the highest concentration used (50%) from the sum of concentration difference, multiplied by mean percentage mortality and divided by the control (100). Results showed that Cyahalothrin exposed to *Pseudomonas* species for 28 days had 30.99%, Chlorpyrifos exposed to *Pseudomonas species* had 12.83 %, Cyahalothrin exposed to *Bacillus* species had 12.77%, Chloropyrifos exposed to *Bacillus species* had 10.77 % (Tables 4.2b to 4.5b and Figure 4.5.). This indicated that Chlorpyrifos exposed to *Bacillus species* had the lowest median lethal concentration (10.77%) and the highest toxic effect while Cyahalothrin exposed to *Pseudomonas species* had the highest median lethal concentration (LC_{50}) and the lowest toxic effect according to the report of Williams and Dilosi (2018); Kpormon and Douglas (2018). The results obtained in this research work revealed that pesticides (Chlorpyrifos and Cyahalothrin) have the ability to inhibit biological processes that are mediated by key environmental microorganisms such as *Bacillus* and *Pseudomonas species* etc in soil. Due to the effect observed on the survival rate of these organisms in this study, it indicates that these pesticides are capable of causing serious environmental pollution which will not only affect the microorganisms and their functions but also the abiotic components of the environment.

Introduction

Pesticides are substances that are meant to control pests, including weeds (USEPA, 2018). The term pesticide includes all of the following: herbicide, insecticides (which may include insect growth regulators, termiticides, etc.) nematicide, molluscicide, piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial, and fungicide (Randall *et al.*, 2014).

Most pesticides are intended to serve as plant protection products (also known as crop protection products) which in general protect plants from weeds, fungi, or insects (Randall *et al.*, 2014).

No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990).

The study of pesticide effects on non-target populations is an accepted strategy to evaluate its associated potential environmental risks. Among non-target populations, soil microorganisms are extremely important, since they play an essential role in nutrient turnover and maintaining generative capacity in agro-ecosystems (Bohlen *et al*., 2002).

The increased use of pesticides in agricultural soils causes the contamination of the soil with toxic chemicals (Muñoz-Leoz *et al*., 2013). When pesticides are applied, the possibilities exist that these chemicals may exert certain effects on non-target microorganisms such as *Pseudomonas* and *Bacillus species* which are of great importance in the soil (Zhao *et al*., 2013). The microbes play an important role in the soil ecosystem (Khan *et al*., 2010), and their functions (Khan *et al*., 2007) are very crucial in nutrient cycling and decomposition (Lorenzo *et al*., 2001).

Materials and Methods

Place of study

The study area was the Rivers State University school farm, Faculty of Agriculture, Rivers State, Nigeria. The University farm is a large area of land that specializes in fish farming, livestock farming, poultry farming and all types of agricultural product farming. Soil samples were collected from 8-10cm depth with a sterile trowel from the plot of land where legumes are cultivated at the Rivers state university agricultural farm, and transferred into sterile polythene bags ensuring they were tied immediately to minimize contamination.

Microbiological analysis

Serial dilution

9ml each of the prepared diluent (normal saline) was dispersed into different test tubes using a sterile pipette. The test tubes were plugged with cotton wool. Both the test tubes and its contents were sterilized in an autoclave at 121℃ for 15 minutes. Tenfold serial dilution for total *Pseudomonas* and *Bacillus* count from soil sample was done by taking 1 gram of soil sample into the autoclaved 9ml test tubes containing diluents, swirled properly for homogeneity to give 10^{-1} dilution. This was repeated for 10^{-2} until dilutions were made up to 10^{-3}. (Cheesebrough, 2006).

Media preparation

Nutrient agar was prepared by weighing 28g of nutrient agar into 1000ml of distilled water in Erlenmeyer flask. The medium was sterilized at 121℃ for 15 minutes using the autoclave at 15psi. The medium was allowed to cool down to 45℃ and 15ml of the meduim was poured into sterile petri dishes.

Centrimide agar was prepared by weighing 45.3g of the centrimide agar and measuring 10ml of glycerol in 990ml of distilled water. The medium was heated with frequent agitation and boiled to completely dissolve the medium before autoclaving at 121℃ for 15 minutes. The medium was allowed to cool and then poured into sterile petri dishes.

Inoculation and incubation of cultures

Inoculation of total *Pseudomonas* and *Bacillus species* was done by aseptically transferring an aliquot (0.1ml) of the dilution of 10^{-3} into properly dried nutrient agar plates and centrimide agar plates for *Bacillus* and *Pseudomonas species* in duplicates, spread evenly using bent glass rod and incubated at

35-37°C for 24hours respectively (Cheesebrough, 2006).

Identification of pure culture

The microorganisms used for this research wok were already identified species of *Bacillus* and *Pseudomonas* isolated from a soil sample.

Preparation of chlopyrifos stock toxicants

The chlopyrifos stock toxicant was prepared based on Manufacturers description (800ml of pesticides into 100 litres of water). This toxicant was prepared with a volume of 8 ml pesticides transferred into 1 litre of distilled water.

Preparation of cyhalothrin stock toxicant

The cyhalothrin stock toxicant was prepared based on manufacturer's prescription (800ml of pesticides into 100 litres of water). This toxicant was prepared with a volume of 8ml, pesticides transferred into 1 litre of distilled water.

Preparation of toxicants (chlopyrifos and cyhalothrin)

The toxicants were prepared aseptically by using different concentrations as: 3.125%, 6.25%, 12.5%, 25% and 50% respectively of the toxicants. These concentrations were obtained aseptically by transferring 0.62ml, 1.25ml, 2.5ml, 5ml, 10ml of the different pesticides stock solution into 19.38ml, 18.75ml, 17.5ml, 15ml, 20ml of sterile distilled water respectively.

Toxicity testing procedure

This test was done in accordance with the method of Williams and Ogolo (10.2018) and Atunanya *et al.,* (11. 2016). Ten millimeter (10ml) of the test organism was added to six plates containing 200g of soil sample and different toxicant concentrations (3.125%, 6.25%, 12.5%, 25% and 50% respectively).

One gram of soil samples was serially diluted to 10^{-3} and an aliquot (0.1ml) from each concentration of soil was then plated out using spread plate techniques.

Inoculation and spreading was done for 1, 7, 14, 21 and 28 days and incubated for 24 hours at room temperature (28±2°C.) after which the colonies on the plate were counted. The formula below was used to estimate the percentage log survival and mortality of the test organisms.

Percentage (%) log survival =log c ÷ log e x100

where
log c=logarithm count in each toxicant concentration
log e=logarithm count in the control (zero concentration)
percentage (%) log mortality=100 - % log survival (Williams and Ogolo 2018).

LC50=LC100 -

$$\in conc.diff x mean \%mortality 1\% \frac{mortality}{\%control}$$

Results and Discussion

Physiochemical analyses results

The results of physiochemical parameters analyzed in this study presented in table 1 below showed that the soil was acidic, deficient in calcium but rich in phosphate.

Toxicity results

The results of toxicity assay carried in this study are presented in Tables 2a to 5b respectively. The tables show the percentage survival and Mortality of the test organisms exposed to various concentrations of the test organisms from day 1 to 28 while Table 2b to 5b show the results of median lethal concentration of the respective pesticides exposed to *Bacillus* and *Pseudomonas species* in soil.

Percentage logarithm survival of the respective organisms used is presented in Figures 1 to 4, while Figure 5 shows the comparative values of the median lethal concentrations of the two pesticides to the two test bacteria.

The table 2b shows the median lethal concentration of Cyahalothrin from the percentage mortality of *Pseudomonas species* in soil. The median lethal concentration LC_{50} of Cyahalothrin in soil was 30.99%.

Table.1 Physiochemical Analysis

S/No.	Parameter	Result
1	pH	5.3
2	Temperature (°C)	26.7
3	Phosphate (Mg/kg)	84.98
4	Magnesium (ppm)	1.51
5	Calcium (ppm)	0.01
6	Potassium (ppm)	1.38
7	Sodium (ppm)	2.29
8	Nitrate (Mg/kg)	2.747.25
9	Sulphate (Mg/kg)	1,101.38

Table.2a Lethal toxicity results of cyahalothrin on *Pseudomonas species* in soil

Time/Conc. (%)	3.125	6.25	12.5	25	50
CONTROL	100	100	100	100	100
Day 1					
% survival	58.37	53.06	51.6	48.57	43.67
% mortality	41.63	46.94	48.4	51.43	56.33
Day 7					
% survival	54.31	53.45	50.00	31.90	23.28
% mortality	45.69	46.55	50.00	68.10	76.72
Day 14					
% survival	74.62	31.82	30.68	11.36	0
% mortality	25.38	68.18	69.32	88.64	100
Day 21					
% survival	0	0	0	0	0
% mortality	100	100	100	100	100
Day 28		0	0	0	0
% survival	0	0	0	0	0
% mortality	100	100	100	100	100

Table.2b Median lethal Concentration (LC50) of Cyahalothrin from Percentage Mortality of *Pseudomonas species* in Soil

Concentration (%)	**% Mortality**	**Mean % mortality**	**Conc. different**	**∑ of Conc. diff. × mean % mortality**
0	**0**	0	0	
3.123	312.7	62.54	3.123	195.31
6.25	361.7	72.34	3.123	225.91
12.5	367.72	73.54	6.25	459.65
25	408.17	81.63	12.5	1020.43
50	433.05		25	
				∑ = 1901.3

$$LC_{50} = LC_{100} - \frac{\sum \text{CONC. DIFF.} \times \text{MEAN \% MORTALITY}}{\text{\% CONTROL}}$$

$$LC_{50} = 50 - \frac{1901.3}{100}$$

$$LC_{50} = 50 - 19.01$$

$$LC_{50} = 30.99\%$$

Table.3a Lethal toxicity results of chlorpyrifos on *pseudomonas* species in soil

Time/Conc. (%)	**3.125**	**6.25**	**12.5**	**25**	**50**
CONTROL	100	100	100	100	100
Day 1					
% survival	65.04	59.35	54.07	51.63	48.78
% mortality	34.96	40.65	45.93	48.37	51.22
Day 7					
% survival	65.31	55.10	52.65	48.57	40.82
% mortality	34.69	44.90	47.35	51.43	59.18
Day 14					
% survival	44.78	39.93	34.33	30.22	25
% mortality	55.22	60.07	65.67	69.78	75
Day 21					
% survival	0	0	0	0	0
% mortality	100	100	100	100	100
Day 28					
% survival	0	0	0	0	0
% mortality	100	100	100	100	100

Table.3b Median lethal Concentration (LC50) of Chlorpyrifos from percentage mortality of *Pseudomonas species* in soil

Concentration (%)	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	0	0	0	
3.123	324.87	64.74	3.123	202.91
6.25	345.62	69.12	3.123	215.87
12.5	358.95	71.79	6.25	448.69
25	369.58	73.92	12.5	923.95
50	385.4	77.08	25	1927
				∑ = 3718.42

$$LC_{50} = LC_{100} - \frac{\sum \text{CONC. DIFF.} \times \text{MEAN \% MORTALITY}}{\text{\% CONTROL}}$$

$$LC_{50} = 50 - \frac{3718.42}{100}$$

$$LC_{50} = 50 - 37.18$$

$$LC_{50} = 12.82\%$$

Table.4a Lethal toxicity results of Cyahalothrin on *Bacillus* species in Soil

Time/Conc. (%)	**3.125**	**6.25**	**12.5**	**25**	**50**
CONTROL	100	100	100	100	100
Day 1					
% survival	57.49	54.66	52.63	47.37	40.48
% mortality	42.51	45.34	57.37	52.63	59.52
Day 7					
% survival	47.62	43.25	39.68	37.30	30.95
% mortality	52.38	57.75	60.32	62.70	69.04
Day 14					
% survival	41.24	37.23	31.75	25.19	17.15
% mortality	58.76	62.77	68.25	74.81	82.85
Day 21					
% survival	36.8	33.6	31.2	27.2	6.8
% mortality	63.2	66.4	68.8	72.8	93.2
Day 28					
% survival	22.5	15	11.25	7.5	0
% mortality	77.5	85	88.75	92.5	100

Table.4b Median Lethal Concentration (LC50) of cyahalothrin from percentage mortality of *Bacillus species* in soil

Concentration (%)	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	**0**	0	0	
3.123	294.4	58.88	3.123	183.88
6.25	317.3	63.46	3.123	198.19
12.5	343.5	68.7	6.25	429.38
25	355.4	71.08	12.5	888.5
50	404.6	80.92	25	2023
				∑ = 3722.95

$$LC_{50} = LC_{100} - \frac{\sum \text{CONC. DIFF.} \times \text{MEAN \% MORTALITY}}{\text{\% CONTROL}}$$

$$LC_{50} = 50 - \frac{3722.95}{100}$$

$$LC_{50} = 50 - 37.23$$

$$LC_{50} = 12.77\%$$

Table.5a Lethal toxicity results of chlorpyrifos on *Bacillus* species in soil

Time/Conc. (%)	3.125	6.25	12.5	25	50
CONTROL	100	100	100	100	100
Day 1					
% survival	51.26	47.29	42.96	61.01	54.15
% mortality	48.74	52.71	57.04	38.99	45.85
Day 7					
% survival	26.86	24.79	37.19	31.40	24.79
% mortality	73.14	75.21	62.81	68.60	75.21
Day 14					
% survival	22.54	19.37	25.35	18.31	16.89
% mortality	77.46	80.63	74.65	81.69	83.11
Day 21					
% survival	18.62	17.24	15.17	0	0
% mortality	81.38	82.76	84.83	100	100
Day 28					
% survival	14.92	12.20	8.47	0	0
% mortality	85.08	87.80	91.53	100	100

Table.5b Median lethal concentration (LC50) of chlorpyrifos from percentage mortality of *Bacillus species* in soil

Concentration (%)	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	**0**	0	0	
3.123	365.8	73.16	3.123	228.48
6.25	379.11	75.82	3.123	236.79
12.5	370.86	74.17	6.25	463.58
25	389.28	77.86	12.5	973.2
50	404.17	80.83	25	2020.85
				∑ = 3922.9

$LC_{50} = LC_{100} - \frac{\sum \text{CONC. DIFF.} \times \text{MEAN \% MORTALITY}}{\text{\% CONTROL}}$

$LC_{50} = 50 - \frac{3922.9}{100}$

$LC_{50} = 50 - 39.23$

$LC_{50} = 10.77\%$

Figure.1 Percentage log Survival of *Pseudomonas* spp Exposed to Various Concentrations of Cyahalothrin

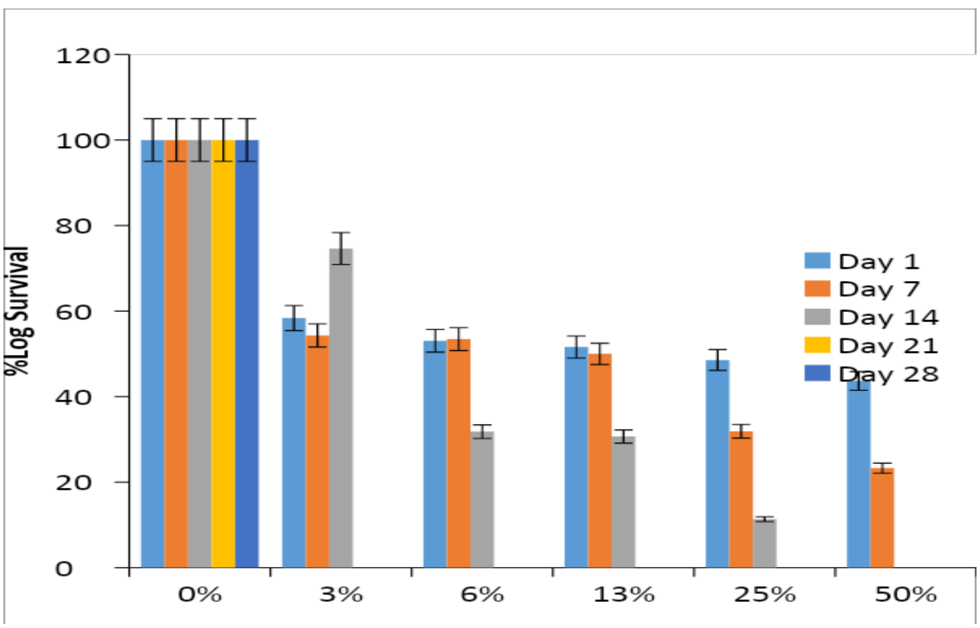


Figure.2 Percentage log survival of *Pseudomonas species* exposed to various concentrations of chlorpyrifos

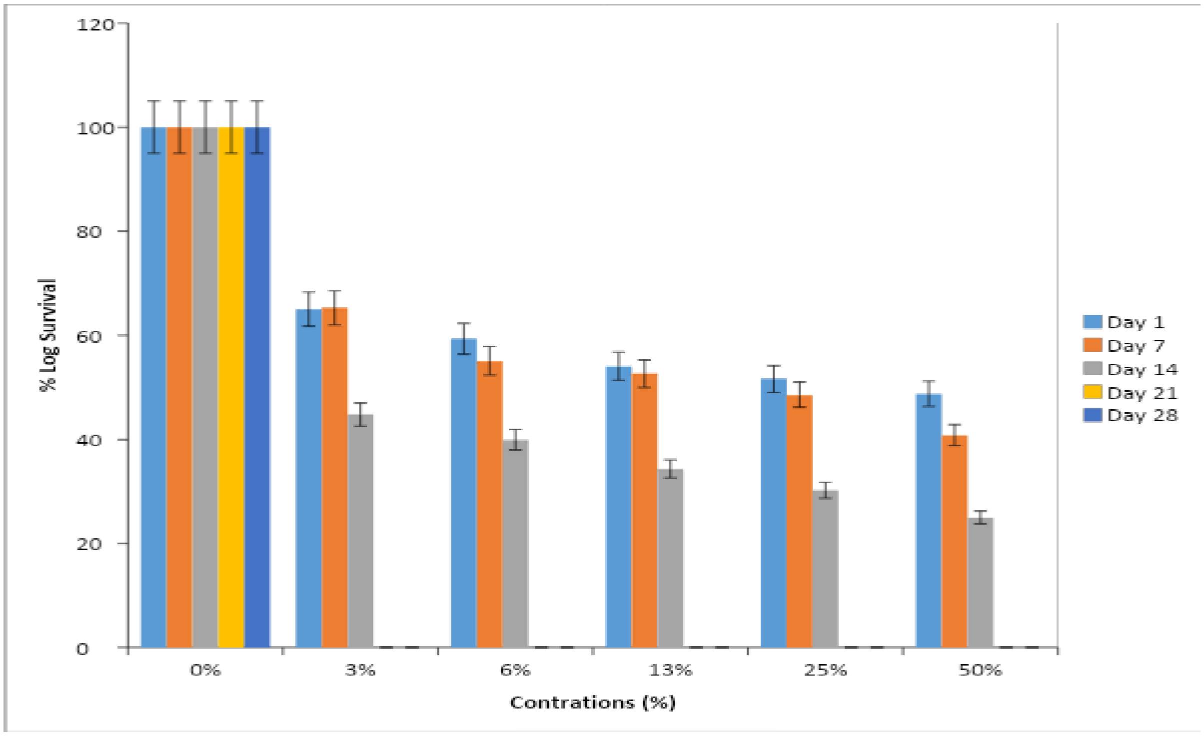


Figure.3 Percentage log survival of *Bacillus species* exposed to various concentrations of cyahalothrin

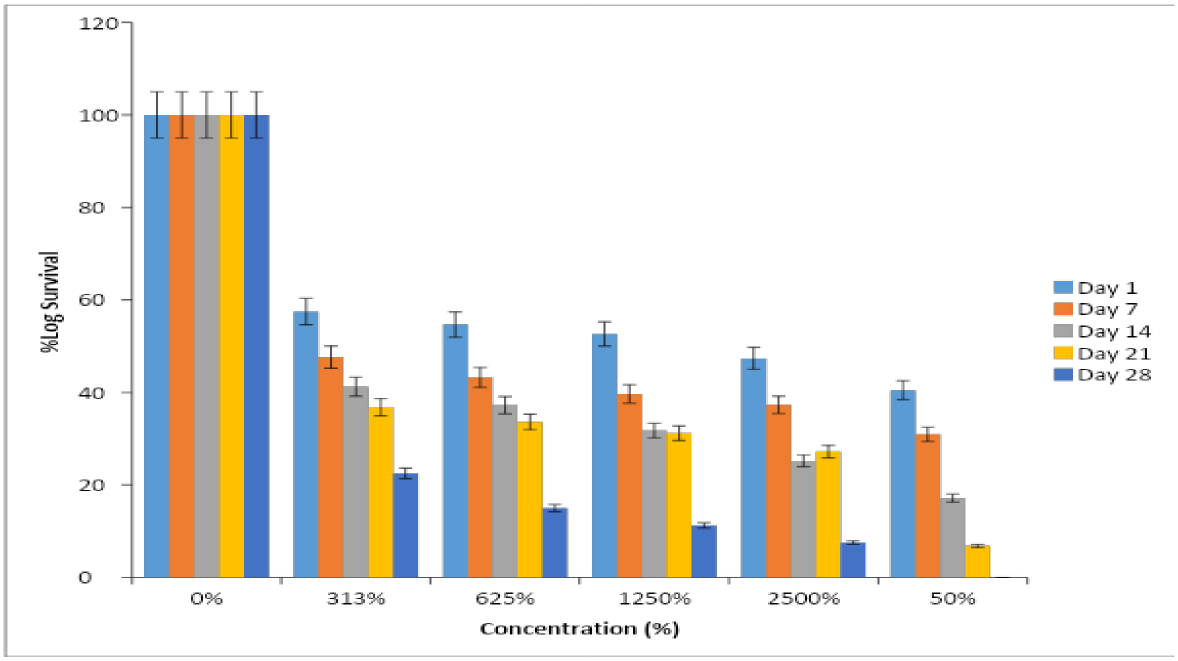


Figure.4 Percentage log survival of *Bacillus species* exposed to various concentrations of chlopyrifos

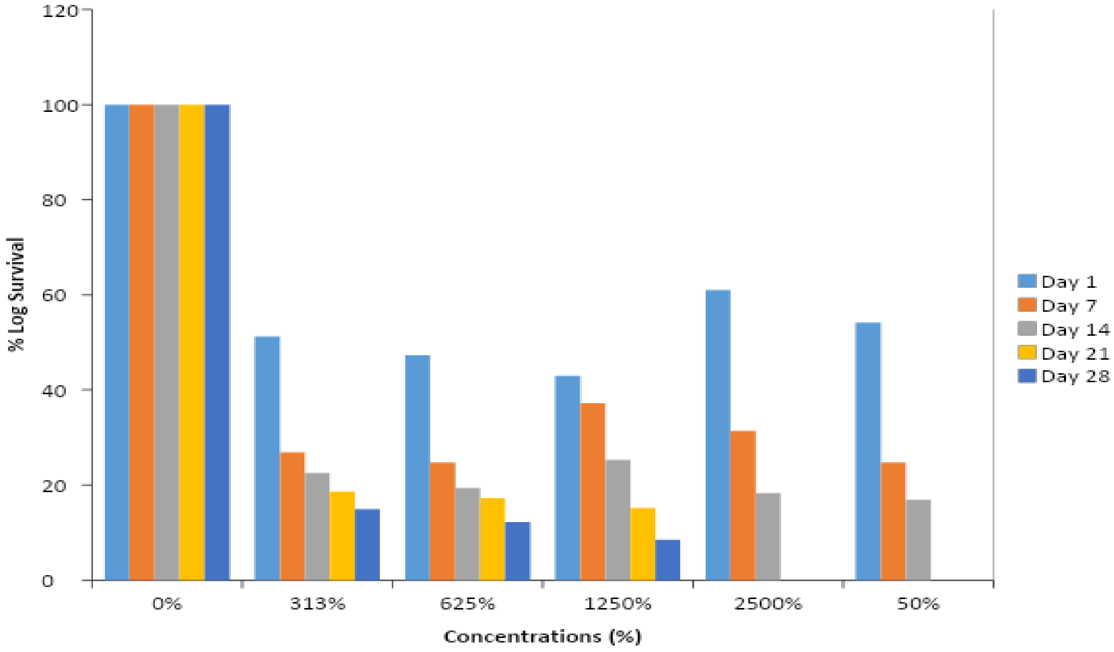
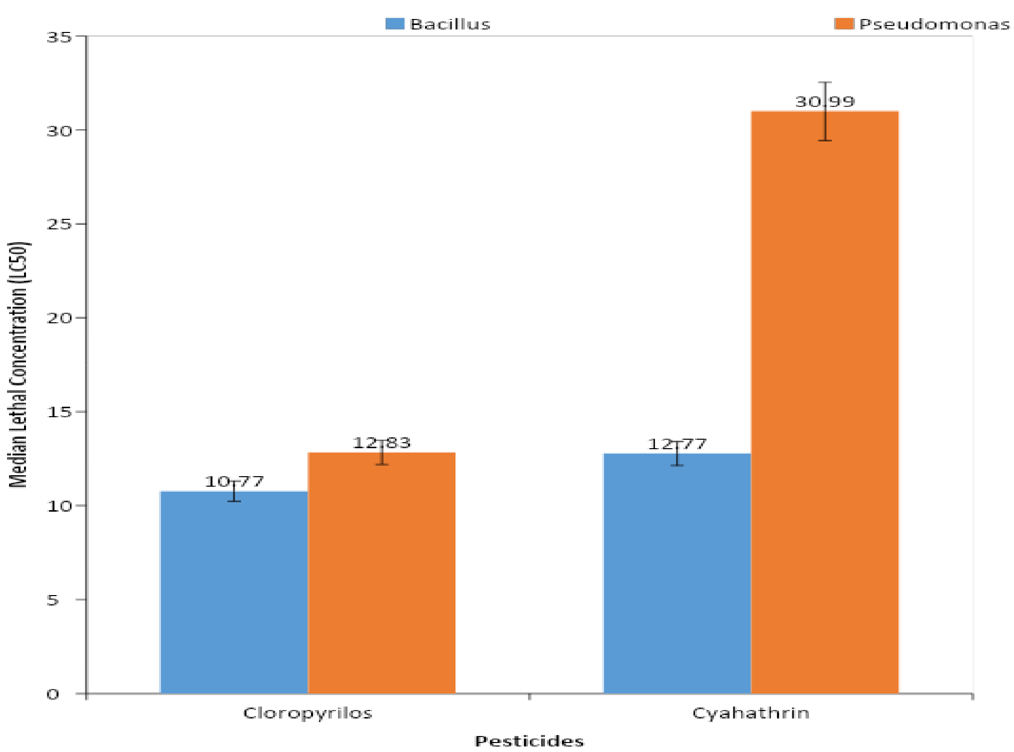


Figure.5 Median lethal concentrations of the pesticides to the test bacteria



The table 3a shows the lethal toxicity of Chlorpyrifos on *Psuedomonas species* in soil.

The table 3b shows the median lethal concentration LC50 of Chlorpyrifos from percentage mortality of *Pseudomonas species* in soil.

Conclusion and recommendation are given below:

The results obtained in this research work revealed that pesticides (Chlorpyrifos and Cyahalothrin) have the ability to inhibit biological processes that are mediated by key environmental microorganisms such as *Bacillus* and *Pseudomonas* species *etc.* in soil. Due to the effect observed on the survival rate of these organisms in this study, it indicates that these pesticides are capable of causing serious environmental pollution which will not only affect the microorganisms and their functions but also the abiotic components of the environment.
Government should set up pre-market regulation requirements, mandating chemical firms to test chemicals for their ability to kill pests so that only the pesticides that are environmentally friendly will be developed further. In this way, pesticides that are environmentally hazardous can be rejected.

Application of pesticides by farmers is as a result of lack of adequate awareness of the side effects, therefore, farmers should revert to the traditional hand hoeing, hand weeding, use of pest resistant seeds and organic farming systems. This will reduce the use of indiscriminate pesticides in the environment.

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