Original Research Article

RESPONSE OF CHEMOLITHOTROPHIC NITROBACTER, NITROSOMONAS TO TOXICITY OF ORGANOPHOSHPHATE AND PYRETHROID PESTICIDES.

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A B S T R A C T

Aim: to investigate the response of *Nitrosomonas* and *Nitrobacter* species to organophosphate and pyrethroid pesticides.

Study design: this study employs experimental design and statistical analysis of data and interpretation.

Place and duration of studies: soil samples were collected from university school farm, rivers state university. Port Harcourt. Nigeria. Samples were transported to the microbiology laboratory of rivers state university immediately for microbiological and toxicity testing. Pesticides was gotten No.4 Ignatius Ajuru University Road, St. John Campus, Aba Road Port Harcourt. The toxicity testing was done for the duration of 7 days interval for 28days respectively at room temperature.

Methodology: standard microbiological techniques were used: toxicity testing procedures were carried out by preparing the pesticides at concentrations of 0%, 3.125%, 6.25%, 12.5%, 25% and 50% and tested on the soil samples for the duration of 1, 7, 14, 21 and 28 days respectively. Samples were serially diluted and cultures were incubated at 35^0C For 18 to 24hours. LC_{50} was determined using SPSS version 2.0

Results: the results indicate that logarithm mortality of *Nitrosomonas* and *Nitrobacter* species increases with increase toxicant concentration and exposure time for pyrethroid pesticide while decreases with increase toxicant concentration and exposure time for organophosphate pesticides. The median lethal concentration LC_{50} of the pesticides increases in the following order: (Note: the higher the LC_{50}, the lower the toxic effect); pyrethroid pesticide on *Nitrosomonas* (53.1%) < organophosphate pesticide on *Nitrosomona* (47.9%), pyrethroid pesticide on *Nitrobacter* (53.5%) < organophosphate pesticide on *Nitrobacter* (47.5%).

Conclusion: The results revealed that different concentrations of the toxicants have both negative and positive effect on the survival rate of the test organisms which shows that the organophosphate pesticide can cause more harm to the environment affecting *Nitrosomonas* and *Nitrobacter* species that plays vital functions in nutrient fixation in the soil environment. While pyrethroid pesticides at appropriate concentrations can stimulate the growth of these organisms there by increasing the rate of nutrient fixation in the soil environment. But also, when these toxicants are misapplied they can cause harm to humans that would consume the crops.

Keywords: pyrethroid pesticide, organophosphate pesticide, toxicity, *nitrosomonas*, *nitrobacter,* nitrification.

1. INTRODUCTION

The tolerance abilities of chemolithotrophic bacteria (nitrifying bacteria) to some commonly used pesticides in the Rivers State University school campus and environment cannot be under emphasized. Basically, the importance of chemolithotrophic bacteria in the biogeochemical cycle / environment has been noted beneficial [1]. Chemolithotrophic bacteria are known for their use of inorganic compounds as electron donor in their nutritional diet, their survival is dependent on the physio chemical condition of its immediate environment [1]. Pesticides are extensively used in agriculture as a part of pest control scheme. Owing to their xenobiotic characteristics, pesticides may adversely affect the proliferation of beneficial soil microorganisms and their associated bio- transformation in the soil. Inactivation of nitrogen-fixing and phosphorus- solubilizing microorganisms is observed in pesticide-contaminated soils, However, a few reports reveal some positive effects of applied pesticides on soil health

2. MATERIAL AND METHODS

Soil samples were collected from university school farm, faculty of agriculture, Rivers state university with sterile trowel at the rhizosphere of legumous plant (mukuna beans) in a sterile polyethene bag and transported to the microbiology laboratory immediately. The used pesticides were gotten from No.4 Ignatius Ajuru University Road, St. John Campus, Aba Road Port Harcourt.

Microbiological Analysis

Isolation of *Nitrosomonas* species

Winogradsky Agar medium composition as modified as modified by [2], was used: Agar Agar 15.0g, $FeSO_4.7H_2O$ 0.4g, Nacl 2.0g, K_2HPO_4 1.0g, $MgSO_4.7H_2O$ 0.5g, and $(NH_4)_2SO_4$ 2.0g were dissolved in 1000ml of distilled water and autoclaved at 121^0C for 15 minutes (psi) after which was allowed to cool to about 40^0C and the medium was poured into Petri- dishes. Then, the medium was solidified before progress to the hot air oven to moisture. One (1) gms of soil was dissolved into 9ml of sterile distilled water and 10 fold serial dilution was done to 10^{-3} and an aliquot from each soil concentration were inoculated unto winogradsky agar and incubate aerobically for 2-3 days at room temperature (30 ± 2^0C), greyish, mucoid, flat colonies revealed pear shaped, and Gram negative of *nitrosomonas*.

Confirmation of *Nitrosomonas* species

Suspected *Nitrosomonas* species were sub cultured on a fresh winogradsky agar medium and transferred into a broth containing Ammonium sulphate and Sodium nitrate and incubated at about (30 ± 2^0C) for 2-3 days. 1ml of sulfanilic acid, dimethylnapthalamine and zinc dust was added was added to the medium after 2days of incubation. Red coloration indicated by nitrate production from ammonia sulphate was a confirmation of *nitrosomonas* species.

Isolation of *Nitrobacter* species

Winogradsky Agar medium composition as modified as modified by [2] was used: Agar Agar 15.0g, $NaNo_2$ 0.05g, Na_2CO_3 1g, Nacl 0.3g, K_2HPO_4 0.5g, $MgSO_4.7H_2O$ 0.02g, $Zncl_2$ 0.03g and $FeSO_4.6H_2O$ 0.02g were dissolved in 200ml of distilled water and autoclaved at 121^0C for 15 minutes (psi) after which was allowed to cool to about 40^0C and the medium was poured into Petri- dishes. Then, the medium was solidified before progress to the hot air oven to moisture. One(1)gms of soil was dissolved into 9ml of sterile distilled water and 10 fold serial dilution was done to 10^{-3} and an aliquot from each soil concentration were inoculated unto winogradsky agar and incubate aerobically for 2-3 days at room temperature (30 ± 2^0C), greyish, mucoid, flat colonies revealed pear shaped, and Gram negative of *nitrobacter.*

Confirmation of *Nitrobacter* species

Suspected *Nitrobacter* species were sub cultured on a fresh winogradsky agar medium and transferred into a broth containing nitrite carbonate medium and incubated at about (30 ± 2^0C) for 2-3 days. 5 drops of Griess illosvay's reagent was added to the medium after 2days of incubation. Absent of purplish colour indicate a positive result for *nitrobacter* species, further confirmation was done by diphenylamine. Cherry red indicated presence of *nitrobacter.*

Preparation stock toxicant

The stock toxicant was prepared based on manufacturer prescription (800ml of pesticides into 100 liters of water). The toxicant was prepared, with a volume of 8ml, pesticides transferred into 1litre of distilled water from which the concentrations were obtained from.

Toxicity test procedures

The toxicants were prepared aseptically by using different concentrations: as 3.125%, 6.25%, 12.5%, 25% and 50% respectively of the toxicant. These concentrations were obtained aseptically by transferring 3.125ml, 6.25ml, 12.5ml, 25ml, and 50ml of the different pesticides stock solution into 96.8ml, 93.75ml, 87.5ml, 75ml, 50ml, of sterile distilled water respectively. The toxicity test procedures was done by using 12 clay pots containing 1.5kg of oven sterilized soil, 10ml of bacteria (*nitrobacter* and *nitrosomonas spp*) was added separately and each toxicant concentration were added separately into different clay pots and a control experiment was done without inoculation of pesticides. One gms of soil sample from all concentrations was serially diluted and an aliquot from 10^{-3} dilution was used for inoculation using spread plate techniques on winogradsky media after 1, 7, 14, 21 and 28 days respectively and was incubated for 2-3 days at room temperature (37 ± 2^{0}C).

. **Toxicity test of bacteria (*nitrobacter* and *nitrosomonas* spp) in pesticides**

The percentage log survival of *Nitrobacter* and *Nitrosomonas* species isolated in the pesticides polluted soils were calculated according to formula used by [3]. The percentage log survival of the bacteria isolates in the soil was calculated by obtaining the log of the count in toxicant concentration, divided by the log of the count in the zero toxicant concentration and multiplying by 100. Thus:

$$\text{Percentage (\%) log survival} = \frac{\text{Log C}}{\text{Log c}} \times 100$$

Where Log C = Logarithm count in each toxicant concentration, log c = Logarithm count in the control (zero toxicant concentration).

Percentage (%) log mortality =100 - % log survival

3. RESULTS AND DISCUSSION

The logarithm counts of *Nitrosomonas* and *Nitrobacter* species revealed the response of these bacteria to organophosphate and pyrethroid pesticides are revealed in Tables 1 and 2 respectively. Percentage logarithm mortality of the counts are presented in the figures below.

The result obtained from this study revealed that pesticides can inhibit nitrification process as well as encourage it by *Nitrobacter* and *Nitrosomonas* species. Similar observation have been reported [4,5,6]. An increase in the percentage logarithm of mortality of *Nitrosomonas* and *Nitrobacter* in in treated with organophosphate pesticide after 28days of exposure to the toxicant concentrations, and an increase in percentage logarithm of survival rate of *Nitrosomonas* and *Nitrobacter* species in soil treated with pyrethroid pesticide after 28days of exposure to the toxicant concentrations were observed. (figs.1to5) respectively. This study also revealed that the toxicant (organophosphate pesticide) is more toxic to the organisms than pyrethroid pesticide. This may be as a result of its chemical composition and at same time its degradability by this organisms. The site of action of any toxicant depends on the nature of the toxicant.

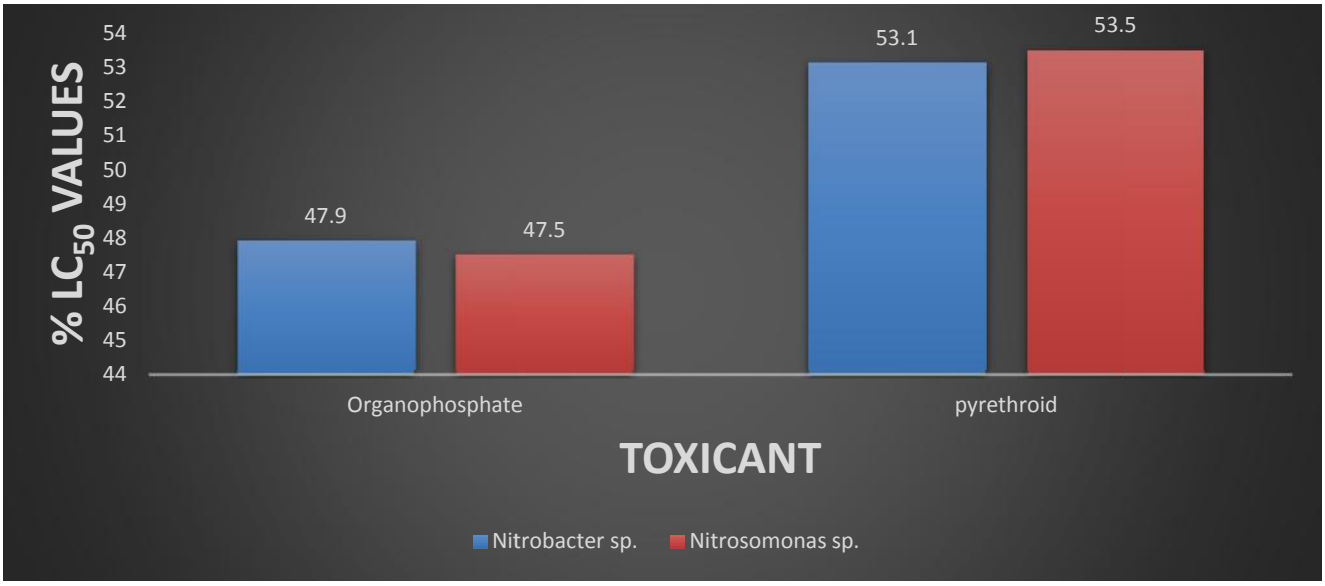


Fig.1. summary of median lethal concentration of pesticides (organophosphate and pyrethoid) on *Nitrosomonas* and *Nitrobacter sp*.

Table 1. log count of *Nitrobacter s*pecies with organophosphate and pyrethroid pesticides

Pyrethroid pesticide + *Nitrobacter*							Organophosphate pesticide + *Nitrobacter*				
CONC/DURATION	1	7	14	21	28	(Days)	1	7	14	21	28
0%	5.68	5.75	5.77	5.81	5.84		6.0	5.91	5.83	5.74	5.72
3.125%	5.72	5.78	5.82	5.85	5.87		5.97	5.86	5.80	5.72	5.68
6.25%	5.74	5.82	5.84	5.88	5.90		5.92	5.79	5.66	5.60	5.57
12.5%	5.76	5.85	5.87	5.89	5.91		5.87	5.70	5.60	5.51	5.43
25%	5.79	5.87	5.89	5.92	5.94		5.83	5.65	5.52	5.50	5.41
50%	5.81	5.94	5.95	5.96	5.97		5.81	5.61	5.48	5.45	5.38

Table 2. log count of *Nitrosomonas* species with organophosphate and pyrethroid pesticides

Pyrethroid pesticide + *Nitrosomonas*							Organophosphate pesticide + *Nitrosomonas*				
CONC/DURATION	1	7	14	21	28	(Days)	1	7	14	21	28
0%	5.74	5.79	5.81	5.87	5.90		6.10	6.0	5.93	5.86	5.83
3.125%	5.77	5.81	5.86	5.89	5.93		6.08	5.98	5.88	5.85	5.79
6.25%	5.79	5.85	5.87	5.91	5.96		5.99	5.88	5.82	5.81	5.76
12.5%	5.86	5.88	5.90	5.92	5.97		5.88	5.81	5.76	5.74	5.71
25%	5.88	5.90	5.93	5.95	5.98		5.80	5.75	5.71	5.66	5.62
50%	5.93	5.97	5.98	5.99	6.01		5.72	5.65	5.63	5.60	5.57

Table 3. Median lethal conc. (LC_{50}) from percentage log mortality of pyrethoid on *Nitrosomonas*

CONC.	**%mortality**	**Mean of mortality**	**Conc. Diff**	**Sum conc.diff. *mean mortality**
0	-	-	-	-
3.125%	-6	-1.2	3.125	-3.75
6.25%	-15	-3	3.125	-9.38
12.5%	-23	-4.6	6.25	-28.75
25%	-28	-5.6	12.5	-70
50%	-40	-8	25	-200 =-311.9

$LC_{50} = 50 - (-) \frac{311.9}{100}$

$LC_{50} = 50+3.1 = 53.1$

Table 4. Median lethal conc. (LC_{50}) from percentage log mortality of pyrethroid on *Nitrobacter*

CONC.	%MORTALITY	MEAN OF MORTALITY	CONC. DIFF.	SUM OF CONC.DIFF* MEAN MORTALITY
0%	-	-	-	-
3.125%	-10	-2	3.125	-6.25
6.25%	-21	-4.2	3.125	-13.13
12.5%	-24	-4.8	6.25	-30
25%	-31	-6.2	12.5	-77.5
50%	-44	-8.8	25	-220
				= -346.9

Median lethal conc (LC_{50}) from percentage log mortality of pyrethroid on *Nitrobacter*

LC_{50} =50-(-) $\frac{346.9}{100}$

50-(-) 3.5 =50 + 3.5

= 53.5

Table 5. Median lethal concentration (LC_{50}) from percentage % log mortality of *Nitrobacter*

CONCENTRATION	%MORTALITY	MEAN%MORTALITY	CONC. DIFF.	SUM OF CON DIFF x MEAN%MORTALITY
0%	-	-	-	-
3.125%	3	0.6	3.125	1.88
6.25%	11.7	2.3	3.125	7.2
12.5%	21.1	4.2	6.25	26.37
25%	26.5	5.3	12.5	66.25
50%	30.1	6.02	25	150.5
				= 252.2

LC50 = 50 – $\frac{252.2}{100}$

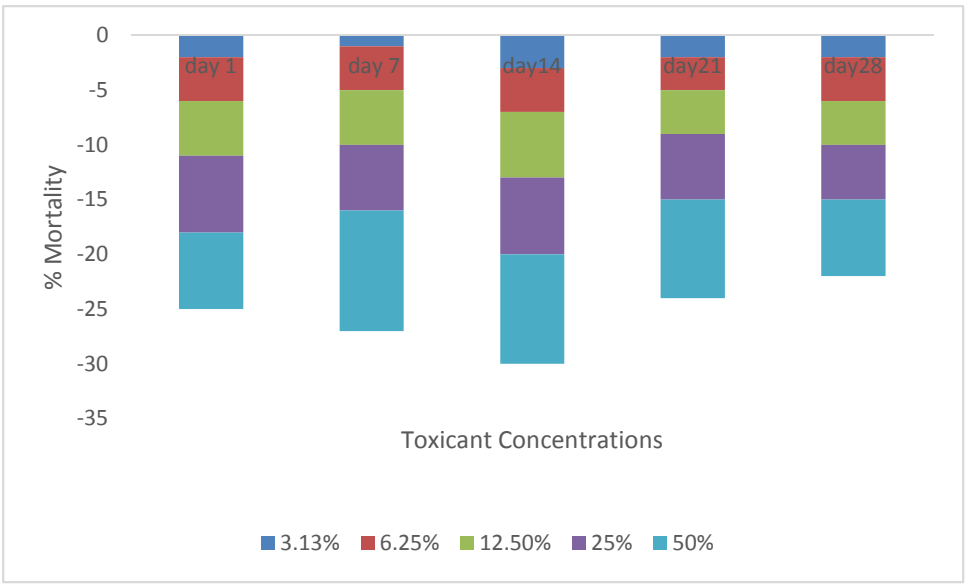
= 50 – 2.5 = 47.5

Table 6. Median lethal concentration (LC50) from %percentage log mortality OF *Nitrosomonas*

CONCENTRATION	%MORTALITY	MEAN OF %MORTALITY	CONC. DIFF.	SUMOF CON.DIF. X MEAN%MORTALITY
0%	-	-	-	-
3.125%	2.6	0.52	3.125	1.63
6.25%	8	1.6	3.125	5
12.5%	14	2.8	6.25	17.5
25%	20.2	4.04	12.5	50.5
50%	26.3	5.3	25	132.5
				= 207.13

LC50 =50 – $\frac{207.13}{100}$

= 50 – 2.07 =47.9



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144 Fig2. % mortality rate of Nittobacter when exposed to pyrethroid pesticide.
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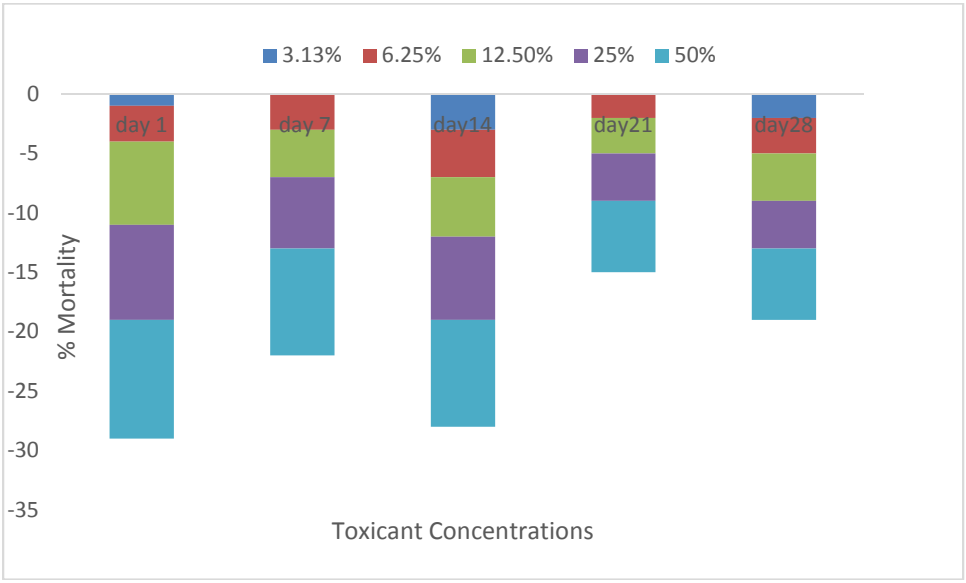

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147 Fig3. % mortality rate of Nitrosomonas when exposed to pyrethroid pesticide
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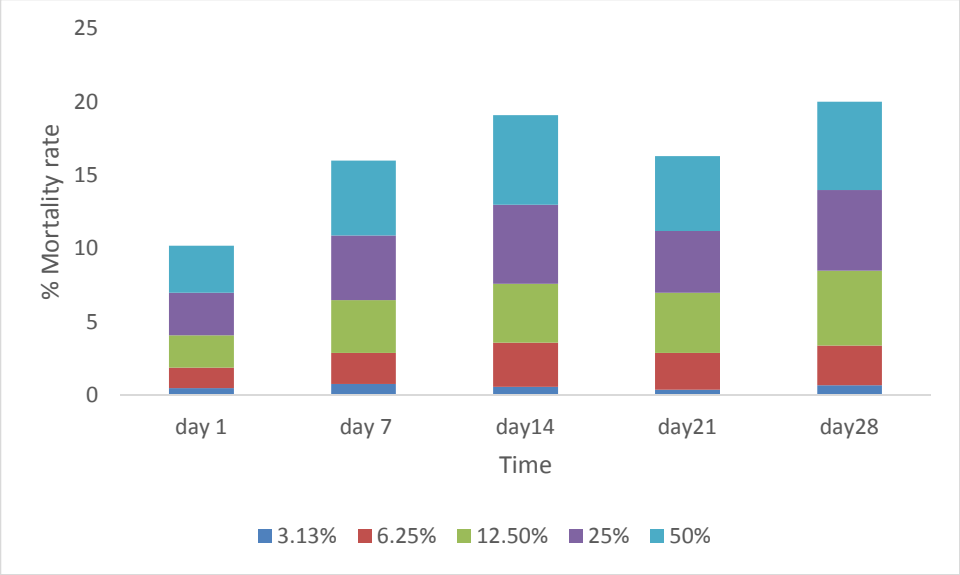

Fig4. % mortality rate of *Nitrobacter* when exposed to organophosphate pesticide

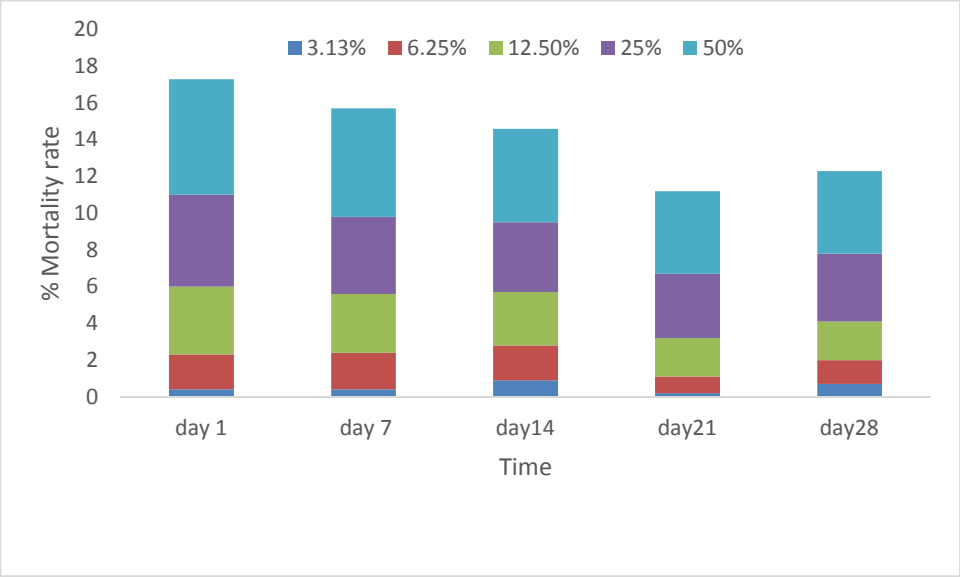

Fig 5: % mortality rate of *Nitrosomonas* when exposed to organophosphate pesticide

The percentage log survival of *Nitrosomonas* and *Nitrobacter* species during 28 days exposure period to soil treated with Organophosphate and Pyrethroid pesticides.(Tables 1 and 2) respectively shows that organophosphate pesticide exhibited little effect on the test organisms than pyrethroid pesticide. This may be due to their chemical composition of the toxicant. The percentage log mortality of *Nitrosomonas* and *Nitrobacter* species during 1, 7, 14, 21 and 28 days exposure periods to the different concentrations of the toxicants reveals that the mortality rate of organophosphate pesticide is higher than that of pyrethroid. Hence the results of this study suggest that organophosphate pesticide causes cell death which resulted in reduction of viable cell counts while pyrethroid pesticide was able to serve as carbon source for these organisms and thereby they were able to utilize them and proliferate which lead to increase in viable cell counts. The reduction of viable cell count of organophosphate pesticide may lead to inhibition of nitrification process during the 28days exposure period. Similar observation was done by [9], while pyrethroid pesticide lead to the increase in nitrification process because the organisms were able to utilize it as their sole carbon source, similar observation was done by [8].

Nitrosomonas and *Nitrobacter sp*. Mortality expressed as median lethal concentration (LC_{50}) was used as indices to monitor toxicity [lucky and d].the sensitivity of these bacteria to the toxicity of the different concentration of the pesticides. The median lethal concentration (LC_{50}) of the pesticides used increased in the following order: (note: the higher the LC50, the lower the toxic effect and vise-visa) pyrethroid on *Nitrosomonas* (53.5%) < organophosphate on *Nitrosomonas* (47.5%), pyrethroid on *Nitrobacter* (53.1%) < organophosphate on *Nitrobacter* (47.9%). Conclusively organophoshate pesticide was more toxic to the organisms, even though most toxic to *nitrosomonas* (LC_{50}= 47.5%) having the most toxic effect while pyrethroid on *nitrosomonas* (LC_{50} =53.5%) having the lowest toxicity effect.

Conclusion and Recommendation

The results revealed that different concentrations of the toxicants have both negative and positive effect on the survival rate of the test organisms which shows that the organophosphate pesticide can cause more harm to the environment affecting *Nitrosomonas* and *Nitrobacter* species that plays vital functions in nutrient fixation in the soil environment. While pyrethroid pesticides at appropriate concentrations can stimulate the growth of these organisms there by increasing the rate of nutrient fixation in the soil environment. But also, when these toxicants are misapplied they can cause harm to humans that would consume the crops.

Therefore, it is recommended that pesticides should be applied according to manufacturer prescription and not misapplied, pyrethroid pesticides should be encouraged.

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