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Response of Chemolithotrophic Nitrobacter, Nitrosomonas to Toxicity of Organophoshphate and Pyrethroid Pesticides

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Authors' contributions

This work was carried out in collaboration between both authors. Author LBD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JOW managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

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 obtained from University 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 period of 28 days at room temperature.
Methodology: Standard microbiological techniques were used: toxicity testing procedures were carried out by preparing a stock culture of the pesticide based on manufactures directions (8 ml into 1000 ml of distilled water) from which the concentrations used for this research work were obtained 0%, 3.125%, 6.25%, 12.5%, 25% and 50% and tested on the soil samples for a period of 28 days.

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Samples were serially diluted and cultures were incubated at 35°C for 18 to 24 hours. 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 play 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 campus and environment cannot be underemphasised. 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 an electron donor in their nutritional diet, their survival is dependent on the physicochemical condition of its immediate environment [1]. Pesticides are extensively used in agriculture as a part of pest control scheme. Due to the xenobiotic characteristics of pesticides, which may have led to adverse effects on the survival of beneficial soil microorganisms and their associated bio-transformation. Inactivation of nitrogen-fixing and phosphorus-solubilising microorganisms have been observed in pesticide-contaminated soils. However, a few reports have revealed some positive effects of applied pesticides on soil health.

2. MATERIALS AND METHODS

2.1 Place of Study

Soil samples were collected from University Farm, Faculty of Agriculture, Rivers State University, Port Harcourt Nigeria. With sterile trowel at the rhizosphere of leguminous 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.

2.2 Microbiological Analysis

2.2.1 Isolation of *Nitrosomonas* species

Winogradsky Agar medium composition as modified by Williams JO and Ogolo CN [2], was used: Agar Agar 15.0 g, $FeSO_4.7H_2O$ 0.4 g, Nacl 2.0 g, K_2HPO_4 1.0 g, $MgSO_4.7H_2O$ 0.5 g, and $(NH_4)_2SO_4$ 2.0 g were dissolved in 1000 ml of distilled water and autoclaved at 121°C for 15 minutes (psi) after which was allowed to cool to about 40°C 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°C), greyish, mucoid, flat colonies revealed pear shaped, and Gram negative of *Nitrosomonas*.

2.2.2 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°C) for 2-3 days. 1 ml of sulfanilic acid, dimethylnapthalamine and zinc dust was added was added to the medium after

2 days of incubation. Red coloration indicated by nitrate production from ammonia sulphate was a confirmation of *nitrosomonas* species.

2.2.3 Isolation of *Nitrobacter* species

Winogradsky Agar medium composition as modified by Williams JO and Ogolo CN [2] was used: Agar Agar 15.0 g, $NaNo_2$ 0.05 g, Na_2CO_3 1 g, Nacl 0.3 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.02 g, $Zncl_2$ 0.03 g and $FeSO_4.6H_2O$ 0.02 g were dissolved in 200 ml of distilled water and autoclaved at 121°C 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°C), greyish, mucoid, flat colonies revealed pear shaped, and Gram negative of *Nitrobacter.*

2.2.4 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°C) for 2-3 days. 5 drops of Griess illosvay's reagent was added to the medium after 2 days of incubation. Absent of purplish colour indicates a positive result for *Nitrobacter* species, further confirmation was done by diphenylamine. Cherry red indicated the presence of *Nitrobacter.*

2.3 Preparation Stock Toxicant

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

2.4 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 3.125 ml, 6.25 ml, 12.5 ml, 25 ml, and 50 ml of the different pesticides stock solution into 96.8 ml, 93.75 ml, 87.5 ml, 75 ml, 50 ml, of sterile distilled water respectively. The toxicity test procedures was done by using 12 clay pots containing 1.5 kg of oven sterilised soil, 10 ml 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 gram 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°C).

2.5 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 Williams JO [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 [4,5,6,7]. An increase in the percentage logarithm of mortality of *Nitrosomonas* and *Nitrobacter* in in treated with organophosphate pesticide after 28 days of exposure to the toxicant concentrations, and an increase in percentage logarithm of the survival rate of *Nitrosomonas* and *Nitrobacter*

species in soil treated with pyrethroid pesticide after 28 days of exposure to the toxicant concentrations were observed (Figs. 1 to 5) 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.

Table 1. Log count of *Nitrobacter* species 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. x 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

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

$LC_{50} = 50+3.1$

$LC_{50} = 53.1$

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

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

$50-(-)\ 3.5 = 50 + 3.5$

$LC_{50} = 53.5$

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

$LC_{50} = 50 - \frac{252.2}{100}$

$= 50 - 2.5 = 47.5$

Table 6. Median lethal concentration (LC_{50}) from %percentage log mortality of *Nitrosomonas*

Concentration	%mortality	mean of %mortality	Conc. diff.	Sum of 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

$LC_{50} = 50 - \frac{207.13}{100}$

$= 50 - 2.07 = 47.9$

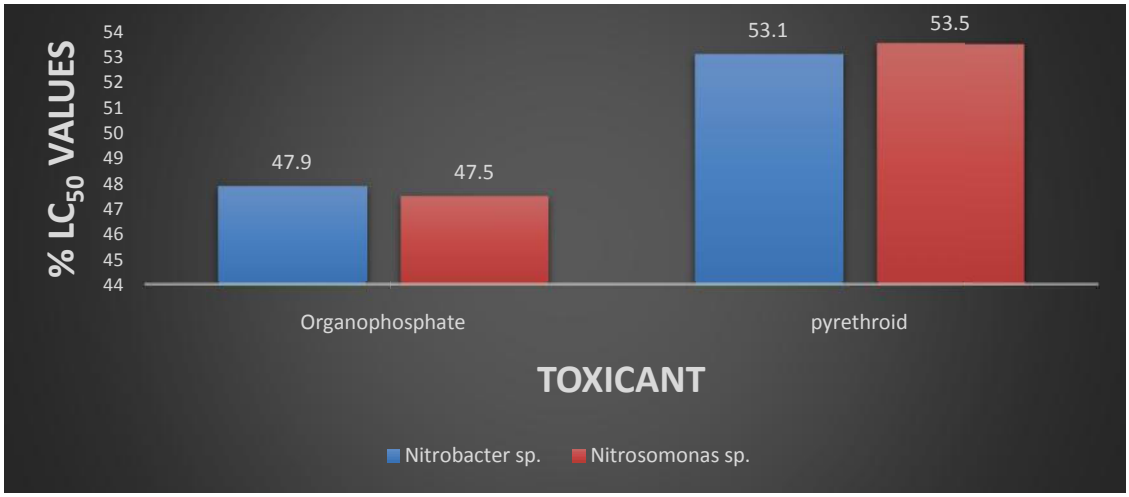

Fig. 1. Summary of median lethal concentration of pesticides (organophosphate and pyrethoid) on *Nitrosomonas* and *Nitrobacter sp*.

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 the 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 utilise 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 28 days exposure period. Similar result was observed by Martinez-Toledo et al. [8], while pyrethroid pesticide lead to the increase in nitrification process because the organisms were able to utilise it as their sole carbon source, similar observation was detected by Sarnaik et al. [9].

Nitrosomonas and *Nitrobacter sp*. Mortality expressed as median lethal concentration (LC_{50}) was used as indices to monitor toxicity [2], the sensitivity of these bacteria to the toxicity of the different concentration of the pesticides. (Tables 3 to 5) shows the median lethal concentrations (LC_{50}) of the pesticides used, which increased in the following order: pyrethroid on *Nitrosomonas* (53.5%) < organophosphate on *Nitrosomonas* (47.5%), pyrethroid on *Nitrobacter* (53.1%) < organophosphate on *Nitrobacter* (47.9%) (Note: the higher the LC50, the lower the toxic effect and vise-visa). Conclusively organophoshate

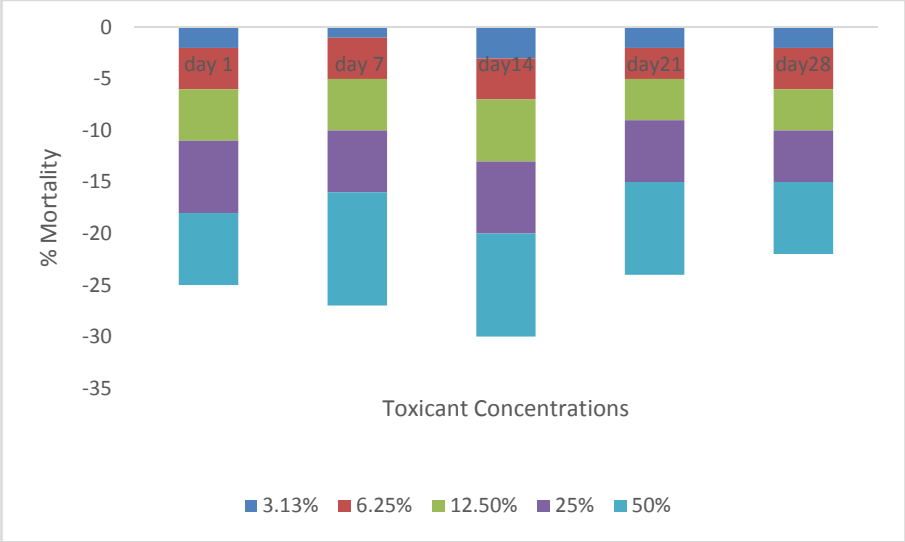

Fig. 2. % mortality rate of *Nitrobacter* when exposed to pyrethroid pesticide

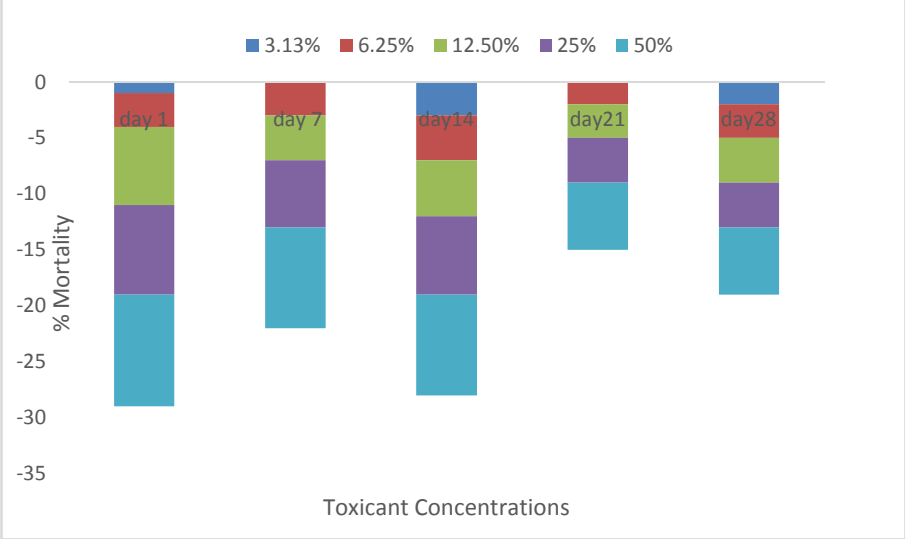

Fig. 3. % mortality rate of *Nitrosomonas* when exposed to pyrethroid pesticide

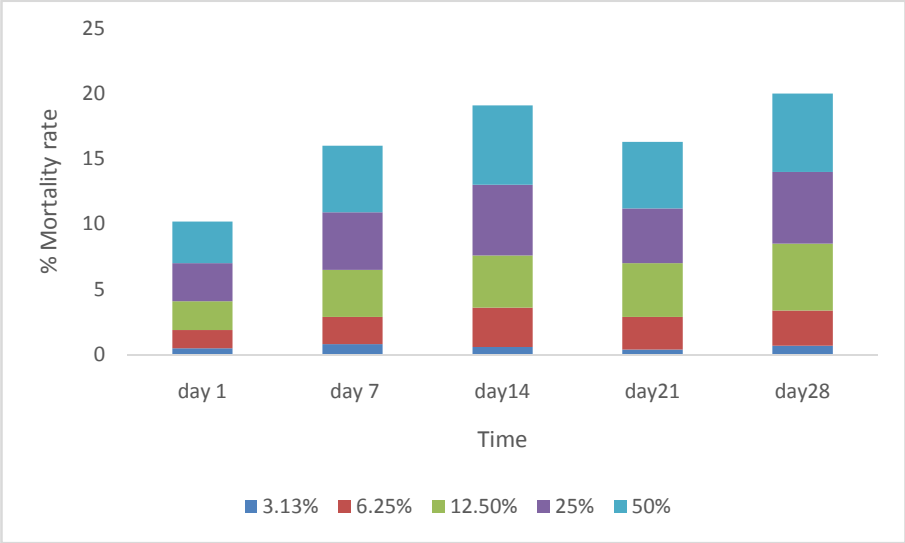

Fig. 4. % mortality rate of *Nitrobacter* when exposed to organophosphate pesticide

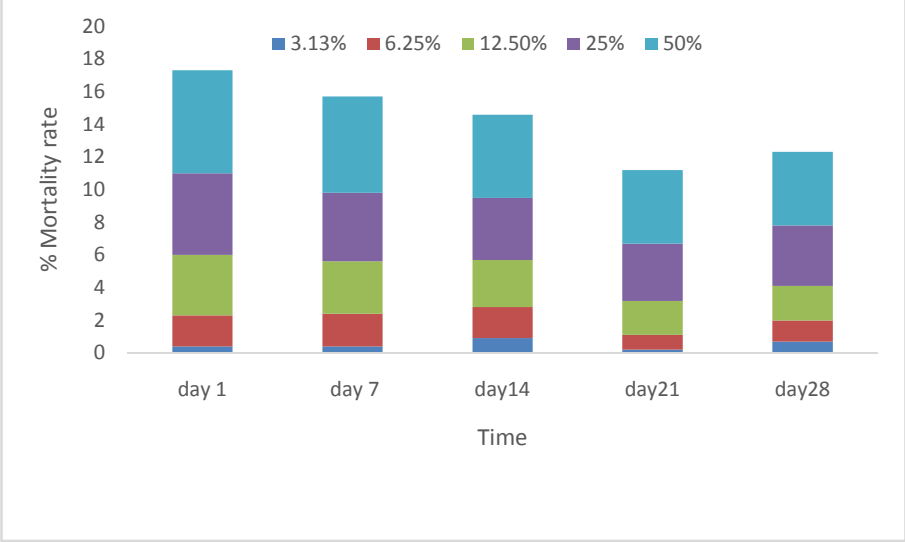

Fig. 5. % mortality rate of *Nitrosomonas* when exposed to organophosphate pesticide

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.

4. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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