IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 13, Issue 2 Ser. I (February. 2019), PP 15-21
www.iosrjournals.org

Tolerance of Nitrobacter and Nitrosomonas Sp to Carbon furan and Cyahalothrin Pesticides in the Soil

Douglas, Salome Ibietela* and Dilosi, Leton Benita
Department of Microbiology, Faculty of Science,
Rivers State University, Nkpolu-Oroworukwo, PMB 5080, Port Harcourt, Rivers State, Nigeria.
Corresponding Author: Douglas, Salome Ibietela

Abstract: *The tolerance of Nitrosomonas and Nitrobacter species to Carbon furan and Cyahalothrin Pesticides in the soil were investigated. This study employs experimental design and statistical analysis of data and interpretation. Soil samples were collected from the school farm, where there is no history of pesticide application. The toxicity testing was done for a period of 28days, at room temperature. Standard microbiological techniques were used: toxicity testing procedures were carried out by preparing pesticides concentrations of: 0%, 3.125%, 6.25%, 12.5%, 25% and 50% and applied to the soil samples for a duration of 28days, taking out samples for analysis at 7days intervals. Median lethal concentration (LC_{50}) was used as indices to monitor the toxicity. The results indicate that logarithm mortality of Nitrosomonas and Nitrobacter species increases with increase in toxicant concentration and exposure time for Cyahalothrin pesticide while, slight decrease was observed with increased toxicant concentration and exposure time for Carbon furan pesticides. The median lethal concentration LC_{50} of the pesticides increased in the following order: (Note: the higher the LC_{50}, the lower the toxic effect); cyahalothrin pesticide on Nitrosomonas (53.1%) < carbonfuran pesticide on Nitrosomonas (48.5%), cyahalothrin pesticide on Nitrobacter (53.5%) < carbonfuran pesticide on Nitrobacter (48.1%). 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 carbon furan pesticides are less tolerated by Nitrosomonas and Nitrobacter species that plays vital functions in nutrient fixation in the soil environment. While cyahalothrin pesticides, if applied at appropriate concentrations, can stimulate the growth of these organisms, increasing the rate of nutrient fixation in the soil environment. However, caution should be applied in the use of these pesticides in order to prevent adverse effect this could have on beneficial soil microbes. This is not only important for crop production, but for the safety of our foods for consumption.*

Keywords*: Cyahalothrin, Carbon furan, pesticide, toxicity, Nitrosomonas, Nitrobacter, nitrification.*

Date of Submission: 23-01-2019 Date of acceptance: 07-02-2019

I. Introduction

Pesticides application contributes greatly to the pollution of the environment. These chemicals are intentionally introduced into the soil environment to control pests, in the area of agricultural. Large scale application of these pesticides produce land pollutions, pesticides leaching into ground water, surface run-off to nearest water bodies, it can also be carried by wind and soil erosion, which to a great extent contributes to the dispersal of these chemicals in the environment far from the source of application. This results in the death of wildlife while some suffer damage to vital functions such as reproductive failure (Johnson *et al*., 2001). The discovery of Dichlorodiphenyltrichloroethane (DDT) residues in seals and penguins far away in un-inhabitable Antarctic region indicates that no part of the environment is free from contamination of pesticides (Chen *et al.* 2001). The applications of pesticides for the control of pest and their activities in the soil have become an issue of concern, since these chemicals are persistent in the environment, could enter food chain, producing devastating effects, as its effect is evident in the environment (Obire and Owaji-Eli, 2014). Biological assay (bioassay) play an important role in the detection of biologically harmful substances and their effects observed as cellular, behavioural, metabolic and genetic damage (Williamson and Nelson, 1983). Toxicity of substances to microorganisms may be viewed through growth inhibition, enzyme activity, oxygen consumption, ATP level and colonies formed on agar plates (Oranusi and Ogugbue, 2002). Use of *Nitrobacter* and *Nitrosomonas* spp as a tool for bioassay was proposed by Williamson and Johnson 1981, where they described the method for the bioassay as simple and the results sensitive. These organisms are chemoautotrophs and derive their chemical energy from the oxidative electron transport chain. These groups of organisms play a very important role in nitrogen removal process especially in wastewater treatment plant, in nitrogen cycle and the overall fertility of the soil and water environment. When it comes to bioassay, microorganisms especially bacteria are the organisms of choice due to their short life cycle, small space required for culturing, ease of handling and lower

cost (Wang and Reed 1983; Williamson and Nelson, 1983). *Nitrobacter* and *Nitrosomonas* spp were selected as the test organisms for toxicity because they exhibited sensitivity for most toxicants greater than that of heterotrophic organisms (Williams and Reeds, 1993). Nitrogen is converted to nitrite after the ammonification process by *Nitrosomonas* which is then oxidized to nitrate by *Nitrobacter*. When toxic substance or waste is present in the environments they can alter this process of nitrogen removal. How toxic a substance is depends on the nature and strength of the substance, which could result in the death of the organism, inhibits nitrification process which continues after removal of the toxic substance. The growth rate of the test organism could be affected.

The long term use of pesticides in food production has resulted in the accumulation of chemical residue in soil which has affected the soil microbial populations by favouring the growth of those organisms that are capable of utilizing them. Studies has shown that pesticides can stimulate or be inhibitory to the growth of certain soil microbes (Obire and Owaji-Eli, 2014). However, some bacteria have tolerated certain pesticides, where the bacteria utilize the pesticide as its source of carbon for growth. (Monkiedje *et al*., 2002).

II. Materials And Methods

Samples collection

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 pesticides used were gotten from a reputable chemical company, by St. John Campus, Aba Road Port Harcourt.

Microbiological Analysis

Isolation of N*itrosomonas* species

Winogradsky Agar medium composition as modified by Williams and Ogolo, (2018), 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 at 15psi after which it was allowed to cool to about 40^0C and the medium was poured into Petri- dishes. One gram (1g) of soil was mixed into 9ml of sterile distilled water and 10-fold serial dilution was done to 10^{-3} and 0.1ml aliquot from each soil dilution was inoculated unto Winogradsky agar, spread using a sterile glass spreader, and incubated aerobically for 3 to 4 days at room temperature (30 ± 2^0C), Grayish, mucoid, flat colonies, and Gram negative, indication 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. One milliliter (1ml) of sulfanilic acid, dimethylnapthalamine and zinc dust 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 Williams and Ogolo, (2018) 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 at 15psi after which it was allowed to cool to about 40^0C and the medium was poured into Petri- dishes. One gram(1g) of soil was mixed into 9ml of sterile distilled water and 10 fold serial dilution was done to 10^{-3} and 0.1ml aliquot from each dilution were inoculated unto Winogradsky agar and incubated aerobically for 2-3 days at room temperature (30 ± 2^0C), greyish, mucoid, flat colonies revealed pear shaped, and Gram negative, suspected *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. Five 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 addition of diphenylamine. Cherry red indicates presence of *Nitrobacter*.

Preparation Cyahalothrin Stock Pesticide

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

Preparation of Carbonfuran Stock Pesticide

The stock pesticide was prepared based on manufacturer's instruction (500g of pesticides into 100 liters of water). The pesticide was prepared, by addition of 5g, into 1litre of distilled water from which the concentrations were obtained.

Toxicity Test Procedures

The pesticides were prepared aseptically by varying the concentrations as follows: as 3.125%, 6.25%, 12.5%, 25% and 50% respectively of the pesticides. Using a measuring cylinder, each was aseptically transferred, that is; 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 pesticides concentration were added separately into the different properly labeled clay pots and a control experiment was done without inoculation of pesticides. One gram of soil sample from all concentrations were serially diluted and 0.1ml aliquot from 10^{-2} to 10^{-3} dilutions was used for inoculation using spread plate techniques on Winogradsky media. The toxicity monitoring was done on days: 1, 7, 14, 21 and 28, respectively, and plates were incubated for 3 to 4days at room temperature (30 ± 2^{0}C). Tolerance level can be measured using the total viable count (TVC) as an index. (Williams and Dilosi, 2018)

.

Toxicity Test of Bacteria (*Nitrobacter* and *Nitrosomonas* spp) in Pesticides

The percentage log survival of the bacteria isolates *Nitrobacter* and *Nitrosomonas* species in the soil was calculated by obtaining the log of the counts in toxicant concentration, divided by the log of the counts in the zero toxicant concentration and multiplied by 100(Douglas *et al*., 2018). 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

III. Results and Discussion

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

The results obtained from this study revealed that pesticides effect could be inhibitory or stimulatory to *Nitrobacter* and *Nitrosomonas* species. An increase in the percentage log mortality of *Nitrosomonas* and *Nitrobacter* in soil amended with carbon furan pesticide during the 28days exposure time to the pesticide concentrations was observed. And an increase in percentage logarithm survival rate of *Nitrosomonas* and *Nitrobacter* species in soil amended with cyahalothrin pesticide during 28days of exposure to the pesticide concentrations were observed, these results are shown in figures 1 to 5. Similar observation have been reported by (Das and Mujahere, 1998). This study also revealed that the pesticide carbon furan is more toxic to the organisms than cyahalothrin pesticide. This may be as a result of its chemical composition and its degradability by these organisms. The site of action of any pesticide depends on the nature of the pesticide.

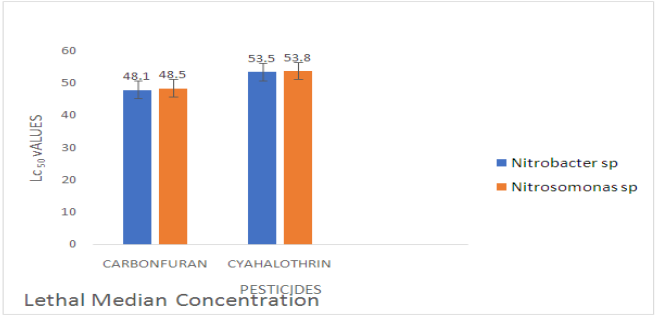

Fig.1: Summary of Lethal Median Concentration of pesticides to *Nitrosomonas* and *Nitrobacter* spp.

Table 1: log counts of *Nitrobacter* species with carbon furan and cyahalothrin pesticides

Cyahalothrin pesticide + *Nitrobacter*							Carbonfuran 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		6.09	5.99	5.94	5.86	5.71
3.125%	5.72	5.78	5.82	5.85	5.87		6.07	5.97	5.87	5.74	5.69
6.25%	5.74	5.82	5.84	5.88	5.90		6.05	5.91	5.84	5.69	5.64
12.5%	5.76	5.85	5.87	5.89	5.91		6.01	5.88	5.83	5.67	5.56
25%	5.79	5.87	5.89	5.92	5.94		5.99	5.83	5.79	5.60	5.54
50%	5.81	5.94	5.95	5.96	5.97		5.94	5.77	5.77	5.59	5.50

Table 2: log counts of *Nitrosomonas* species with Carbon furan and Cyahalothrin pesticides

Cyahalothrin pesticide + *Nitrosomonas*							Carbon furan 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		6.12	6.06	5.95	5.92	5.85
3.125%	5.77	5.81	5.86	5.89	5.93		6.11	6.02	5.91	5.84	5.78
6.25%	5.79	5.85	5.87	5.91	5.96		6.09	6.0	5.83	5.78	5.71
12.5%	5.86	5.88	5.90	5.92	5.97		6.06	5.85	5.76	5.65	5.55
25%	5.88	5.90	5.93	5.95	5.98		5.95	5.85	5.76	5.65	5.55
50%	5.93	5.97	5.98	5.99	6.01		5.93	5.77	5.70	5.63	5.50

Table 3: Median lethal conc. (LC_{50}) from percentage log mortality of cyahalothrin 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 cyahalothrin 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 = -384.9

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

$LC_{50} = 50-(-) \frac{384.9}{100}$

50-(-) 3.8 =50 + 3.8

= 53.8

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

CONCENTRATION	%MORTALITY	MEAN%MORTALITY	CONC. DIFF.	SUM OF CON DIFF x MEAN%MORTALITY
0%	-	-	-	-
3.125%	4.4	0.88	3.125	2.75
6.25%	8.1	1.62	3.125	5.1
12.5%	11.2	2.24	6.25	14
25%	14.5	2.9	12.5	36.25
50%	17.5	3.5	25	87.5
				= 145.6

$LC_{50} = 50 - \frac{145.6}{100}$

$50 - 1.46 = 48.5$

Table 6. Median lethal concentration (LC_{50}) from %percentage log mortality OF carbon furan on *Nitrosomonas*

CONCENTRATION	%MORTALITY	MEAN OF %MORTALITY	CONC. DIFF.	SUMOF CON.DIF. X MEAN%MORTALITY
0%	-	-	-	-
3.125%	4.2	0.84	3.125	2.6
6.25%	8.4	1.68	3.125	5.3
12.5%	12.4	2.48	6.25	15.5
25%	19.3	3.86	12.5	48.3
50%	23.2	4.6	25	115
				= 186.7

$LC_{50} = 50 - \frac{186.7}{100}$

$= 50\text{-}1.87 = 48.13$

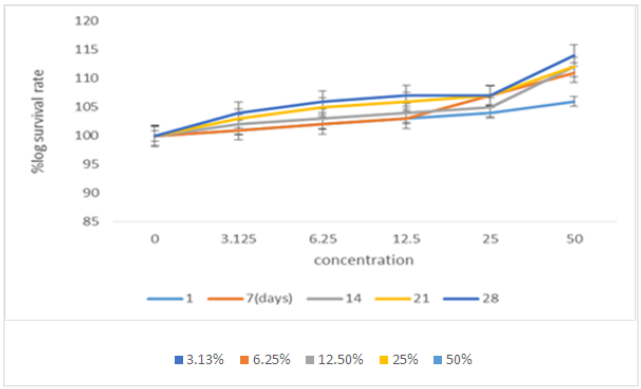

Fig2. % Survival rate of *Nitrobacter* when exposed to Cyahalothrin pesticide.

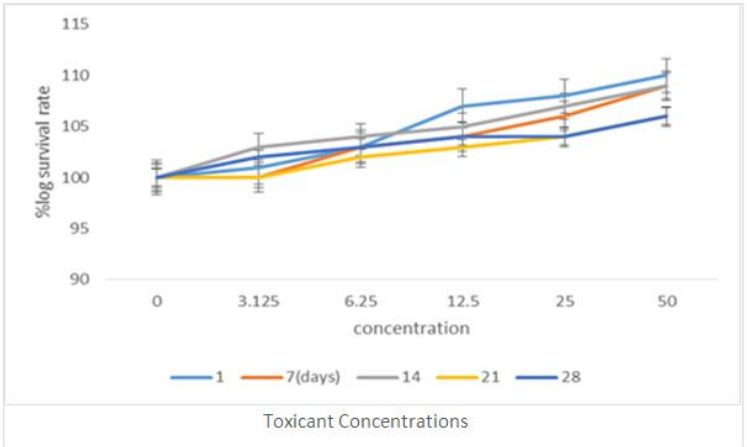

Fig.3: % Survival rate of *Nitrosomonas* when exposed to Cyahalothrin pesticide

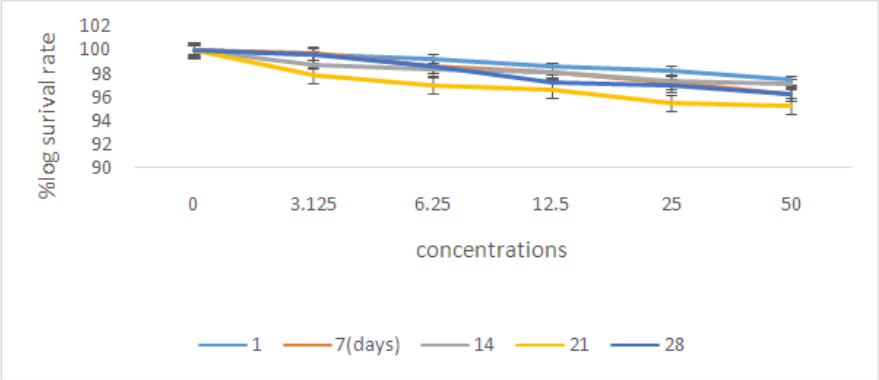

Fig.4: % Survival rate of *Nitrobacter* when exposed to Carbonfuran pesticide

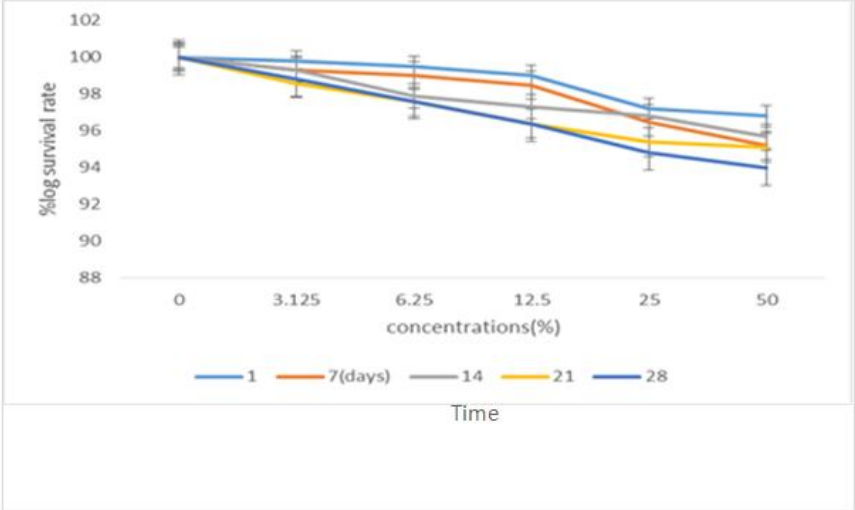

Fig. 5: % Survival rate of *Nitrosomonas* when exposed to Carbonfuran pesticide

The percentage log mortality of *Nitrosomonas* and *Nitrobacter* species during 28 days exposure period to soil amended with Carbon furan and Cyahalothrin pesticides.(Tables 1 and 2), shows that Carbon furan pesticide exhibited slight effect on the test organisms than Cyahalothrin pesticide. This may be due to the chemical composition and molecular structure of the pesticide. Obire and Owaji-Eli, 2014, stated that the effect of different pesticides on soil microorganisms depends on the composition of the pesticide which affects their diversity, due to their xenobiotic nature. The percentage log Survival of *Nitrosomonas* and *Nitrobacter* species during the 28days exposure periods to the various concentrations of the toxicants reveals that the survival rate of cyahalothrin pesticide is higher than that of carbon furan. Hence, the results of this study reveals that carbon furan pesticide caused inhibitory effect on the organisms which resulted in reduction of viable cell counts. while cyahalothrin pesticide was stimulatory, which means it served as carbon source for these organisms and since they were able to utilize them and proliferate which lead to increase in viable cell counts. The reduction of viable cell counts of carbon furan pesticide may lead to inhibition of nitrification process (Obire and Owaji-Eli, 2014). Similar observation was done by (Das and Mukherjee, 2000) while cyahalothrin pesticide lead to the increase in growth of the organisms, which in turn will increase nitrification process because the organisms were able to utilize it as their sole carbon source, similar observation was done by (Williams and Dilosi, 2018).

Nitrosomonas and *Nitrobacter sp*. mortality expressed as median lethal concentration (LC_{50}) was used as indices to monitor toxicity (Kpormon and Douglas, 2018). The median lethal concentration (LC_{50}) of the pesticides used increased in the following order: (note: the higher the LC_{50}, the lower the toxic effect and vise-visa) cyahalothrin on *Nitrosomonas* (53.5%) < carbon furan on *Nitrosomonas* (48.1%), cyahalothrin on *Nitrobacter* (53.1%) < carbon furan on *Nitrobacter* (48.8%). Which means that organophoshate pesticide was more toxic to the organisms, *Nitrosomonas* (LC_{50}= 48.1%) sp suffered the most toxic effect while, cyahalothrin on *Nitrosomonas* (LC_{50} =53.5%) having the least toxicity effect.

IV. Conclusion and Recommendation

The results revealed that the concentrations of the toxicants used in this study on these organisms have both negative and positive effect on the survival rate of the test organisms which shows that the carbon furan pesticide can cause more harm to soil microorganisms, affecting *Nitrosomonas* and *Nitrobacter* species that plays vital roles in nutrient fixation in the soil environment. Cyahalothrin pesticides at appropriate concentrations can stimulate the growth of these organisms there by increasing the rate of nitrogen fixation in the soil environment.

Therefore, it is recommended that pesticides should be applied according to manufacturer's instruction and not misapplied, while the use of cyahalothrin pesticides should be encouraged.

Pesticides contain different active ingredients which could be toxic to farmers, hence, during application proper PPE should be worn.

Competing interests

Authors have declared that no competing interests exist.

References

[1]. Chen, S. K., Edwards, C. A., and Subler, S. (2001). Effect of fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations

[2]. *Soil Biology. Biochemistry.* 33, 1971–1980.

[3]. Das, A. C., and Mukherjee, D. (1998a). Insecticidal effects on soil microorganisms and the Biochemical processes related to soil fertility. *World Journal of Microbiology. Biotechnology*. 14, 903– 909.

[4]. Das, A. C., and Mukherjee, D. (2000b). Influence of insecticides on microbial transformation of Nitrogen and phosphorus in Typic Orchragualf soil. *Journal Agricultural and Food Chemistry*. 48, 3728–3732.

[5]. Douglas, S. I., Nrior, R. R. and Kpormon, L. B.(2018) Toxicity of Spent Phone Batteries on Microflora in Marine, Brackish and Freshwater Ecosystems. *Journal of Advances in Microbiology*. 12(2): 1-10,

[6]. Johnsen, K., Jacobsen, C. S., and Torsvik, V. (2001). Pesticides effects on bacterial diversity in agricultural soils—A review. *Biology. Fertility. Soils* 33, 443–453.

[7]. Kpormon, L. B. and Douglas, S. I. (2018) Comparative Ecotoxicological Assay of E- waste (Phone Batteries) on Some Aquatic Micro Flora. *Journal of Advances in Biology & Biotechnology* 17(2): 1-10.

[8]. Monkiedje, A., Ilori, M. O., and Spiteller, M. (2002). Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. *Soil Biology.*

[9]. *Biochemistry*. 34, 1939–1948.

[10]. Obire and Owaji-Eli, J. 2014 impact of various concentrations of some pesticides on soil bacteria and fungi ISCEST Journal 1 1139- 145

[11]. Oranusi, N. A. and C. J. Ogugbue (2002) Toxic Response of *Nitrobacter* to Orange 11 and Direct Blue 71. *Journal of applied science and Environmental Management*. 6(1): 87 – 90.

[12]. Kpormon, L.B. and Douglas, S. I. (2018). Ecotoxicological assay of E-waste on some aquatic microflora.*JABB.*40421, 17(2): 1- 10.

[13]. Wang, W. and P. Reed (1983) *Nitrobacter* Bioassay for aquatic toxicity. Illinois State water Survey, Peoria, USA.

[14]. Williamson, K. J. and O. G. Johnson (1981) A bacterial bioassay for assessment of wastewater toxicity. *Water Research* 5(15), 383 – 390.

[15]. Williamson, K. J and P. O. Nelson (1983) Bacterial Bioassay for Level 1 Toxicity Assessment. United States Environmental Protection Agency, Chicago.

[16]. Williams, J. O. and Ogolo, C. N. (2018). Response of Aspergillus species to the toxicity domestic detergent's. *Nigeria journal of Microbiology*. 32(1),4334-4343.

[17]. Williams, J.O. and Dilosi, L. B. (2018). Response of Chemolithotrophic Nitrobacter, Nitrosomonas to toxicity of organophosphate and pyrethroid pesticides. *Asian Journal of Biology*. 7(1), 1-8.

Douglas, Salome Ibietela. "Tolerance of Nitrobacter and Nitrosomonas Sp to Carbon furan and Cyahalothrin Pesticides in the Soil." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 13.2 (2019): 15-21.