COMPARATIVE TOXICITIES OF THREE AGRO-INSECTICIDE FORMULATIONS ON NITRIFYING BACTERIA

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ABSTRACT: The toxicity of three agro-insecticides - Lindane, Diazinon and Carbofuran formulations- on *Nitrobacter* and *Nitrosomonas* sp. were investigated. Changes in populations and activities of bacteria isolated from soil samples in the Niger Delta area were monitored following their exposure to different concentrations of the agro-insecticides for four hours. Results of toxicity studies revealed that the median lethal (LC_{50}) and effective (EC_{50}) concentrations of both *Nitrobacter* and *Nitrosomonas* sp. generally decreased with increase in exposure time. The LC_{50} of the three insecticide formulations for both *Nitrobacter* and *Nitrosomonas* sp. decreased thus: Carbofuran > Diazinon > Lindane ($p<0.05$). Four- hour LC_{50} of 417.00, 478.70, 1085.40 mg/l (*Nitrobacter* sp.*)* and 290.20, 259.00, 1018.30 mg/l (*Nitrosomonas* sp.) were obtained for Lindane, Diazinon and Carbofuran, respectively. However, the EC_{50} of the three insecticide formulations for both bacteria were not significantly different ($p> 0.05$). Four- hour EC_{50} of 53.31, 55.28, 66.00 mg/l (*Nitrobacter* sp.*)* and 59.31, 37.59, 51.34 mg/l (*Nitrosomonas* sp.) were obtained for Lindane, Diazinon and Carbofuran, respectively. The study also revealed that *Nitrosomonas* sp. was more sensitive to Lindane and Diazinon than *Nitrobacter* sp. although both bacteria exhibited similar sensitivity Carbofuran. The results suggest that autotrophic transformation by nitrifying bacteria in soil may be hindered following contaminations with these agro-insecticide formulations.

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1.0 INTRODUCTION

Pesticides are one of the major groups of chemicals used worldwide. They are commercially presented in thousands of different chemicals and used in agriculture, public health, forestry, fish and game management. Pesticides can be defined as those chemicals employed by man to destroy or inhibit life forms, which he has decided are a nuisance (Higgins and Burns, 1975).

Agricultural development successes, in Nigeria, are not without the use of insecticides. The insecticides are basically used either in the storage of grains or protecting insecticides that are commonly used in Nigeria include: the chlorinated hydrocarbons (e.g Lindane, Aldrin, etc.); organophosphates (e.g Monocrotophos, Dicholvos, Diazinon, Pirimiphos methyl, etc.); carbamates (eg. Carbofuran, Carbosulfan, Carbaryl, etc.) and the synthetic pyrethroids (eg. Cypermethrin, Cylhalothrin, etc.).

Many authors, although, have reported the brodegradation of some pesticides by microorganisms (Sweeny, 1969; Flashinski and Lichtenstein, 1974; Okpokwasili and Nwosu, 1990), pesticides may influence microbial population directly or indirectly (Higgins and Burns, 1974). Thus, this paper is aimed at determining the relative toxicities of three agro-insecticide formulations on nitrifying bacteria isolated from soils of the Niger Delta Area in Nigeria.

2.0 Materials and Methods

2.1 Isolation and characterization of nitrifying bacteria

Nitrobacter sp. and *Nitrosomonas* sp. were isolated from the soil samples using the method employed by Okpokwasili and Odokuma (1996a,b) and Colwell and Zambruski (1972), respectively.

2.2 Preparation of diluents for toxicity tests

Sodium nitrite (0.25mg $NaNO_2$/l Winogradsky broth) and ammonium sulphate (5.0mg $(NH_4)_2SO_4$/l Winogradsky broth) diluents for *Nitrobacter sp.* and for *Nitrosomonas sp*., respectively, were employed. The diluents were sterilized at 121^oC and 15 psi for 15 minutes.

2.3 Preparation of insecticide concentrations for *Nitrobacter* toxicity tests

For the determination of the median lethal concentration (LC_{50}), toxicant concentrations of 100 mg/l, 300 mg/l, 400 mg/l and 500 mg/l for Lindane /Diazinon and 200 mg/l, 400 mg/l, 600 mg/l, 800 mg/l and 1000 mg/l for Carbofuran were employed. The median effective concentrations (EC_{50}), of the three insecticides were determined from toxicant concentrations of 20mg/l, 40mg/l, 60mg/l, 80mg/l and

100mg/l. A control experiment consisting of the $NaNO_2$ diluent only (without toxicant) was set up.

2.4 Preparation of insecticide concentrations for *Nitrosomonas* toxicity tests

Insecticide concentrations of 100, 150, 200, 250 and 300 mg/l, (Lindane and Diazinon); and 200, 400, 600, 800 and 1000mg/l (Carbofuran) were used for the determination of the median lethal concentration (LC_{50}) while 20, 40, 60, 80 and 100 mg/l of the three test insecticides were employed for the median effective concentrations (EC_{50}), respectively. A control experiment consisting of the $(NH_4)_2SO_4$ diluent only (without toxicant) was also set up.

2.5 *Nitrobacter* and *Nitrosomonas* acute toxicity tests

To each of the toxicant concentrations (90ml) in 250ml volumetric flask, 10ml of bacterial (*Nitrobacter* and *Nitrosomonas*) standard inoculum was aseptically inoculated. Nitrite content was determined (APHA, 1998) and plates containing Winogradsky media were immediately inoculated by spread plate techniques (Okpokwasili and Odokuma, 1996a,b). This was followed by nitrite determinations and spread plate inoculations from the various toxicant concentrations after 1h, 2h, 3h, and 4h incubation intervals. Plates were incubated at room temperature (28 ± 2^oC) for 72 hours. Percentage nitrite utilization (for *Nitrobacter*) and nitrite accumulation (for *Nitrosomonas*) were plotted against toxicant concentration and the median effective concentrations (EC_{50}) were determined using the probit analysis. The percentage inhibition of bacterial growth (log survival) was plotted against toxicant concentration and the median lethal concentration (LC_{50}) value was calculated using the probit analysis. All results were subjected to the analysis of variance (ANOVA) as reported by Finney (1978).

3.0 Results Analysis

3.1 Median lethal concentration (LC_{50}) of Lindane, Diazinon and Carbofuran for *Nitrobacter* and *Nitrosomonas sp.*

The LC_{50} for 1, 2, 3, and 4 h exposure periods of *Nitrobacter sp.* and *Nitrosomonas* sp. to the test insecticides are presented in Table 1and 2. The LC_{50} values decreased with increase in exposure time for the test insecticides. Four- hour LC_{50} of 417.00, 478.70, 1085.40 mg/l (*Nitrobacter* sp.*)* and 290.20, 259.00, 1018.30 mg/l (*Nitrosomonas* sp.) were obtained forLindane, Diazinon and Carbofuran, respectively. One-way analysis of variance (ANOVA) showed that the effects of the insecticides on *Nitrobacter* sp.and *Nitrosomonas* sp. were significantly differently at the probability level of 0.05. The order of toxicity of the insecticides to *Nitrobacter sp.* was: Lindane > Diazinon > Carbofuran.

Table 1: Median lethal concentrations (LC_{50}) of the test insecticides for *Nitrobacter sp*. during a 4h exposure period.

Time, h	Lindane	Diazinon	Carbofuran
	Mean LC_{50} values (mg/l)		
1	520.20	670.10	1236.50
2	633.20	794.40	1280.80
3	633.20	563.70	1146.40
4	417.00	478.70	1085.40

Table 2: Median lethal concentrations (LC_{50}) of the test insecticides for *Nitrosomonas sp.* during a 4h exposure period.

Time, h	Lindane	Diazinon	Carbofuran
	Mean LC_{50} values (mg/l)		
1	324.00.	326.40	1205.20
2	353.20	389.20	1372.60
3	319.00	288.50	1176.10
4	290.20	259.00	1018.30

3.2 Median effective concentrations (EC_{50}) of Lindane, Diazinon and Carbofuran for *Nitrobacter* and *Nitrosomonas sp.*

The EC_{50} values of the three insecticides on *Nitrobacter* sp.and *Nitrosomonas* sp. during 1, 2, 3 and 4 h exposure periods are presented in Table 3 and 4. The data reveals that the EC_{50} values of the insecticides decreased with increase in time of exposure. Four- hour EC_{50} of 53.31, 55.28, 66.00 mg/l (*Nitrobacter* sp.*)* and 59.31, 37.59, 51.34 mg/l (*Nitrosomonas* sp.) were obtained for Lindane, Diazinon and Carbofuran formulations, respectively. However, analysis of variance (ANOVA) revealed that the different inhibitory effects exerted by the various insecticides on nitrite utilization were not significant at 0.05 level of probability.

Table 3: Median effective concentrations (EC_{50}) of the test insecticides for *Nitrobacter sp*. during a 4h exposure period

Time, h	Lindane	Diazinon	Carbofuran
	Mean EC_{50} values (mg/l)		
1	96.03	117.33	97.30
2	76.60	86.96	97.20
3	64.16	68.86	79.88
4	53.31	55.28	66.00

Table 4: Median effective concentrations (EC_{50}) of the test insecticides for *Nitrosomonas sp*. during a 4h exposure period

Time, h	Lindane	Diazinon	Carbofuran
	Mean EC_{50} values (mg/l)		
1	95.92	199.94	144.07
2	74.75	104.13	93.58
3	77.00	72.95	66.77
4	59.31	37.59	51.34

4.0 Discussion

The results of toxicity studies showed that the toxicity of the Lindane, Diazinon and Carbofuran formulations on *Nitrobacter* sp. and *Nitrosomonas* sp. depended on the contact time and insecticide concentrations. The LC_{50} decreased with increase in exposure time for all the test insecticides (Table 1 and 2). Lal and Saxena (1982) have reported that increasing concentrations of organochlorine insecticides, reduced total viable count of marine bacteria while Hicks and Corner (1973) reported that treatment of *B. subtilis* with DDT resulted in alteration of membrane lipid composition. Inhibition of cell division in *S. cerevisae* by chlordane, heptachlor, aldrin and dieldrin has also been reported (Nelson and William, 1971). Data analyses showed that the median lethal concentrations (LC_{50}) of the three insecticides for *Nitrobacter* sp. and *Nitrosomonas* sp. decreased thus: Carbofuran > Diazinon > Lindane ($p<0.05$). It also indicated that Lindane within the exposure time limit, inhibited growth of *Nitrobacter* sp. and *Nitrosomonas sp*. more than Diazinon and Carbofuran. Lang and Cai (2009) in their study observed that chlorothalonil at the field rate had a slight inhibitory effect on one soil only out of six. Conversely, chlorothalonil at higher rates inhibited nitrification significantly in all soils.

Similarly, the EC_{50} values for *Nitrobacter* sp. and *Nitrosomonas* sp. decreased with increase in exposure time for all the test insecticides (Table 3 and 4). The decrease in EC_{50} and LC_{50} with time may be attributed to increased water solubility with time. Statistical analyses at 95% confidence limit showed that the median effective concentration (EC_{50}) of the three insecticides for *Nitrobacter* sp. and *Nitrosomonas* sp. were not significantly different. The low EC_{50} exhibited by the insecticides indicated that interference with enzyme activity was a mode of action of the three insecticides. The inhibition of enzyme activities by insecticides has been documented. Jejuna and Dogra (1978) observed that aldrin at 100 µg/l and 200 µg/l inhibited the enzyme responsible for the metabolism of pentose and tricarboxylic acid cycle intermediates in *Rhizobium* sp. The activities of succinate dehydrogenase and reduced nicotimamide adenine dinucleotide (NAD) dehydrogenase in *B. subtilis* and *E. coli* were inhibited by chlordane (Trudgill and Widdus, 1970; Widdus *et al.*, 1971).

Comparison of sensitivities of populations of *Nitrobacter* sp. and *Nitrosomonas* sp. to the three insecticides reveals that *Nitrosomonas* sp. was more sensitive to Lindane and Diazinon than *Nitrobacter* sp. while both bacterial populations showed similar sensitivities to Carbofuran during the exposure period. Additionally, the analysis revealed that the effects of the three insecticides on both nitrite oxidations by *Nitrobacter* sp. and ammonia oxidation by *Nitrosomonas* sp. were similar. The work of Odokuma and Oliwe (2003) indicated that xylene was more toxic to *Nitrosomonas* sp. than *Nitrobacter* sp. Although both bacteria may have similar cell wall morphology as Gram-negative rods (Holt *et al*., 1994), the difference in response of these bacteria to the insecticides may be due to genetic differences (Patrick *et al.*, 1991).

Comparison of the LC_{50} and EC_{50} of the three insecticides for *Nitrobacter* sp. and *Nitrosomonas* sp. revealed that the EC_{50} values were significantly lower than the LC_{50} values. This indicated that the EC_{50} determination was more sensitive than the LC_{50} determination. This observation is in conformity with earlier reports of Okpokwasili and Odokuma (1994). Rapidity in achieving result is another advantage of the EC_{50} determination over LC_{50} determination.

Conclusion

The results obtained from this study suggest that autotrophic transformation by nitrifying bacteria which enhances soil fertility (hence significant crop production) may be hindered in an ecosystem polluted with these insecticide formulations, as nitrification processes will be reduced.

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