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ECOTOXICOLOGICAL STUDIES OF PESTICIDE FORMULATIONS ON SOIL SENTINEL'S MICROFLORA (*NITROSOMONAS* AND *NITROBACTER* SPP.) AND MESOFAUNA (*EPHYRIODRILUS* SPP.)

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Abstract: The ecotoxicological effect of pesticide formulations at the concentration range of half of the normal field rate (0.5FR), normal field rate (FR), 2FR, 4FR and 8FR respectively on representative microflora and mesofauna soil sentinels was investigated. Inhibition effects over the 1, 2, 3 and 4 hr period of exposure in Winogradsky media were observed for microflora. Avoidance responses were observed for mesofauna in pesticide spiked soils after a 48 hr exposure period. Generally, there was a corresponding decrease in mesofauna population with increase in pesticide concentrations. The LC50 values after 4 hrs of exposure indicated that endosulfan (4.37 μg/l) and paraquat (10.02 μg/l) formulations had more toxic effect on *Nitrosomonas* sp. Cypermethrin and glyphosate showed more toxic effects on Nitrobacter sp. with LC50 values of 10.21 μg/l and 10.50 μg/l respectively. *Ephyriodrilus* spp. were observed to avoid all the pesticide treated soils at different concentrations as compared to pesticide free soils with less than 20 % observed in the soil spiked with 4FR and 8FR for endosulfan and cypermethrin and 8FR for glyphosate and paraquat. Pesticide formulation at the concentration above the recommended rate could adversely affect the soil organisms and affect their important ecological functions.

Keywords: *Ephyriodrilus, Nitrobacter, Nitrosomonas*, Pollution, Sentinels.

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INTRODUCTION

Worldwide agriculture system has substantially relied on pesticide application due to their positive effect on pest management and consequential increase in the agricultural production. Pesticides have considerably contributed to the successes in agriculture practices through improvement in agricultural productivity, control of vector-borne diseases, improvement in the quality of food and aesthetic value of the environment (Okafoagu *et al*., 2017; Ma *et al*., 2021). However, the possibility of pesticide to cause serious pollution in the environment had been a serious concern among the environmental stakeholders. Apart from potential risks that were associated with direct exposure to pesticide during application; evidence has shown that the pesticide application could have non-target effect on the non-intending organisms and the ecosystem at large (Hashimi *et al*., 2020; Koc *et al*., 2020). Report had shown that the proportion of pesticide reaching the intended target organisms might be less than 1 %; while the remaining percentage were reported to contaminate other environmental entities such as soil, air and water (Sujatha *et al*., 2021; Ezeani *et al*., 2022).

Soil is a dynamic ecosystem that harbors a variety of living organisms which include a vast array of microflora, macroflora and fauna (Alaekwe and Abba, 2022; Pulleman *et al*., 2022). Soils are the most significant sink for all environmental pollutants such as pesticide compounds released into the environment by humans' activities and also a medium that control the fate of pollutant through its adsorption-desorption processes (Lunagariya *et al*., 2020; Muhammad *et al*., 2021).

The biological based approaches of soil monitoring such as assessment of impact of chemical compounds on sentinel organisms have become a major important method for soil quality assessment (Kammenga *et al*., 2000). Sentinel species are biological indicators that are used to evaluate potential hazards the presence of pollutant compounds could cause within an ecosystem and the possible effects on the health of the populations. Evidence had shown that the sentinel organisms have strong tendency to respond to the presence of environmental pollutant and the degree of pollution could be evaluated from the possible effect of such pollutant on physiology changes and population response of such organism (Amadi *et al*., 2020).

Laboratory based ecotoxicology tests had been considered as an important preliminary step in environmental risk assessment due to their rapid observable results and possibility of quantifying risks on the exposed organisms (Alves *et al*., 2013). Ecotoxicological evaluation of xenobiotic chemical compounds on soil dwelling sentinel flora and fauna can provide insightful information of the effect of such pollutants on the structure and behavioural response of such sentinel organisms (Singh *et al*., 2021). Furthermore, impact of pollutant on habitat function of a particular sentinel species can also be deduced from ecotoxicological studies (Candello *et al*., 2018).

Various forms of soil flora and fauna had been reported to be a good indicator of soil pollution and thereby represent an ideal sentinel of environmental pollutants. Examples include culturable microbial population, bacteria taxa with well-defined ecological roles such as ammonia-oxidizing genera, the nitrogen-fixing *Rhizobium* and the methane-oxidizing genera as well as soil invertebrates such as earthworm (Lopez *et al*., 2002; Srinivasulu *et al*., 2017).

Microorganisms have been the major driving force of ecosystem sustainability through their vast and diverse metabolic tendency (Pandey and Singh, 2004). They are important to soil health due to their integral role in biogeochemical cycles and ecosystem sustainability (Parkinson and Coleman, 1991; Schafer *et al*., 2007). They also play some key roles in nutrient cycling and energy flow and provide information on the impact of agricultural practices and any external perturbation in the soil system (Shannon *et al*., 2002; Li and Chen, 2004). They are considered as the living pool of organic matter and thereby play the pivotal roles in soil's nutrient balance and maintenance. Their favourable and dynamic thermo-chemical reactions enable them to control major ecosystem services such as biotransformation of dead organic matter. Likewise, microbes, being in direct contact with the soil matrices, were considered as ideal indicators for soil pollution monitoring (Aynalem *et al*., 2021). Furthermore, possible assessment of pollutants' effect on soil sentinel microbes is an important area in the field of ecotoxicology due to the direct link between the soil microbial community and the functional ecosystem services that are necessary for stability and sustainability of the ecosystem.

Soil invertebrates could represent good sentinel organisms for ecotoxicological evaluation of soil pollution due to their direct contact with soil components, as compared to many vertebrates that are indirectly exposed through food chain (Kammenga *et al*., 2000; Candello *et al*., 2018). Among soil invertebrates, earthworms are relevant sentinels of environmental monitoring because of their role in soil functional services (Ezeani *et al*., 2022). They also have the tendency to show observable behavioural responses as a result of the possible effect of the pollutant on their physiological and biochemical properties (Candello *et al*., 2018). Earthworms are macroscopic invertebrates belong to the Oligochaeta group of Phylum Annelida having true coelom and show antioxidant activities (Deswal *et al*., 2020; Verma and Prakash, 2020).

As they inhabit in soil, play important roles in soil health status by facilitating the decomposition of dead organic debris by soil microbes and providing aeration to the soil matrices through their burrow habit (Ansari and Ismail, 2012). Earthworms have been reported to be suitable sentinels of soil pollution because they constitute a greater proportion of biomass of terrestrial invertebrates and their high relative abundance in the soil system. Likewise, their relatively low tolerance to chemical compounds makes them suitable sentinels for ecotoxicological studies.

In Nigeria, reports had shown that most of the farmers that applied pesticide into their farmland had no prior knowledge of the possible effect of such toxic xenobiotic compounds on the environment (Atuanya *et al*., 2018). The common traditional approach to soil pollution assessment which relied on comparison of the evaluated pollutant concentration in the soil samples with specific pollutant threshold values could not provide information of deleterious effects of such pollutant on soil biota (Parmar *et al*., 2016). Furthermore, it has been identified that monitoring the types and quantities of toxic substances in the environment has been an exhaustive and problematic task. Thus, the application of biological-based techniques in the environmental approach of pollutant assessment is highly encouraged. Such a biological-based approach is application of sentinel species in pollution impact analyses in the environment. Thus, this research is on the ecotoxicological studies of commonly used pesticides on selected soil biological sentinels.

MATERIALS AND METHODS

Pesticide compounds used for ecotoxicological studies were two insecticides formulation (endosulfan, and cypermethrin) and two herbicides formulation (glyphosate and paraquat). The respective concentrations for experimental studies were evaluated using the formula described by Zain *et al.* (2013) as given below:

$$X\mu g/kg \text{ soil} = \frac{\text{Recommended field application rate (g a.i. / ha)}}{\text{Amount of a.i.in formulation (g a.i./ l) x 450 l/ha}} \times \frac{1000000\ \mu g}{1 kg}$$

Where;

"g a. i" represent the gram of active ingredients present in pesticide formulation.

"ha" represents the hectare; l represents litre

The corresponding concentrations (μg/kg) as calculated from field application rate (FR) for the treatments range of 0.5FR, FR, 2FR, 4FR to 8FR for each of the respective pesticide formulations were:

- Endosulfan: 2.75, 5.50, 11.00, 22.00, 44.00
- Cypermethrin: 3.13, 6.25, 12.50, 25.00, 50.00
- Paraquat dichloride: 2.22; 4.44; 8.88; 17.76; 35.52
- Glyphosate: 3.33, 6.66, 13.32, 26.64, 53.28

The nitrifiers (*Nitrosomonas* sp. and *Nitrobacter* sp.) represent the sentinel microflora for ecotoxicological assessment and were isolated from the soil samples collected from Kwara State University Teaching and Research Farm, Malete, Nigeria. While, the epigeic earthworms (*Ephyriodrilus* spp.) represent the sentinel macrofauna and were collected from farmland in Malete, Kwara State, Nigeria.

The representative soil sentinel microflora (*Nitrosomonas* sp. and *Nitrobacter* sp.) were isolated using standard media as described by Atuanya *et al.* (2016). The *Nitrosomonas* sp. was isolated using Winogradsky medium phase 1 ($(NH_4)_2SO_4$, 2.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, O.5 g; NaCl, 2.0 g; $FeSO_4.7H_2O$, 0.4 g; $CaCO_3$ 0.01g). While *Nitrobacter* sp. was isolated using Winogradsky medium phase II ($NaNO_2$, 0.1 g; Na_2CO_3, 1.0 g; NaCl, 0.5 g; $FeSO_4.7H_2O$, 0.4 g).

Each of the media components were aseptically introduced into the flask containing 1000 ml distilled water and 15 g of agar were added. The media were sterilized in autoclave at 121 ^{0}C for 30 minutes and allowed to cool to about 45^{0}C before aseptically poured into sterile Petri dishes.

The respective Winogradsky agar plates were aseptically inoculated with 0.1ml of 10^{-1} dilution of soil suspension using spread plate technique. All the inoculated plates were incubated under aerobic conditions at room temperature (28+ 2 ^{0}C) for 72 hours. Pure colonies of each of the sentinel microflora were obtained by repeated streaking of developed discrete colonies onto new agar plates. The stock cultures were prepared by inoculating the discrete pure colony from the respective agar plates into slants and stored at 4 ^{0}C.

The standard inoculum of sentinel microflora was prepared by inoculating the Winogradsky broth media with the inoculum collected from stock culture and incubated on a shaker at room temperature for 72 hours. The inoculum was then standardized by using 0.5 McFarland turbidity standards (Gayathiri *et al.*, 2018).

The acute toxicity test of the pesticide was carried out on *Nitrosomonas* sp. and *Nitrobacter* sp. in Winogradsky media containing the respective concentration of each of the pesticide formulations, while the blank media were used as control. In 250 ml volumetric flask, 10 ml of the standardized inoculum (*Nitrobacter* and *Nitrosomonas* spp.) was aseptically inoculated into 90 ml pesticide treated Winogradsky broth. The viable counts of the respective pesticide treated media were carried out with pore plate technique at time intervals of 1hr, 2hr, 3hr and 4hr respectively for viable.

The percentage inhibitions of *Nitrosomonas* sp. and *Nitrobacter* sp. were determined using the following formula (Atuanya and Tudararo-Aherobo, 2015; Atuanya *et al.*, 2018):

$$\% \text{ inhibition} = \frac{N(\text{control}) - N(\text{sample})}{N(\text{control})} \times 100$$

Where:

$N_{(control)}$ = Number of colonies (cfu/ml) from the control sample.

$N_{(sample)}$ = Number of colonies (cfu/ml) from the treatment sample

The percentage inhibition of bacterial growth (log survival) was plotted against pesticide treatment concentrations and the median lethal concentration (LC_{50}) value was calculated using the probit regression analysis in excel 2016 Microsoft package.

The earthworms' avoidance behaviour towards the pesticide-treated soils was studied according to the International Standard Organization for chemical toxicity test guidelines, as modified by Latha and Basha (2019). Sexually matured, fully clitellate adult earthworm specimens were used for this test. They were collected by digging and hand picking from subsurface litters of the worm cast and immediately transferred to the laboratory for identification according to the method described by Paoletti (1999) The *Ephyriodrilus* spp. were later selected for the avoidance test. The composite soil samples used for ecotoxicology avoidance test on *Ephyriodrilus* spp were collected from Kwara State University Teaching and Research Farm, Malete, Nigeria with total organic carbon content value of 29.0 %, phosphorus content of 9.6 g/kg, pH (H_2O) value of 7.1 and moisture content of 18.3 % (Aborisade *et al.*, 2021).

Five treatment concentrations (i.e., 0.5FR, FR, 2FR, 4FR and 8FR) of each of the respective pesticides were applied to composite soil samples. Three replicates of each pesticide treated soils were placed in plastic square chambers with 500 mL capacity (13 cm × 13 cm × 5 cm high) for each dose of treatment. The chambers were divided in two equal parts and 500 g of each soil moistened and maintained at about 60% water holding capacity during the previous week were placed in each side of the chambers. The treatment was applied to the soil in just one side of the chambers (T1, T2, T3, T4 or T5) and the other side remained pesticide-untreated-control soil (To).

Adult earthworms (*Ephyriodrilus*) with wet weight range between 343 mg to 457 mg were selected as the test specimen. The systems remained overnight in fume hood for solvent evaporation, the divider was then removed, and ten adult earthworms were placed all together in the slit in the middle of each of the chambers. All the chambers were closed with perforated plastic film to allow air circulation, and maintained at approximately 22°C under continuous light for 48 h.

At the end of the test period, the counting of the specimen worms was done on each side of the chambers. The avoidance behaviour of the earthworms to the different soil treatments was calculated by counting the average number of earthworms in each treated soil compartment and compared with the average number of worms in the untreated control soil.

The percentage avoidance was evaluated using the following formula as described by Latha and Basha (2019).

$$\%A = [(C - T)/N] \times 100$$

Where:

%A = percentage avoidance; C = number of worms in the control (To) condition.

T = number of worms in each dose in the same soil (i.e., T1 or T2 or T3 or T4 or T5 or T6); and N = total number of worms.

RESULTS AND DISCUSSION

The results on inhibitory effect of pesticide formulations were presented in Table 1. For endosulfan, at one hour (1 hr) period of observation, the rate of inhibitions were 3.1 %, 7.2 %; 17.1 %, 25.6 % and 32.4 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 9.5 %, 18.9 %; 47.6 %, 53.4 % and 73.0 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 25.8 %, 38.5 %; 62.5 %, 73.2 % and 78.9 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 37.0 %, 53.9 %; 75.9 %, 83.4 % and 90.0 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

For cypermethrin, at one hour (1 hr) period of observation, the rate of inhibitions were 3.0 %, 4.3 %; 10.6 %, 20.9 % and 43.7 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 9.2 %, 6.9 %, 20.8 %, 41.6 % and 62.4 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 12.7 %, 13.7 %; 40.6 %, 64.8 % and 72.4 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 19.5 %, 25.2 %; 54.1 %, 72.4 % and 77.5 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

For glyphosate, at one hour (1 hr) period of observation, the rate of inhibitions were 1.5 %, 5.3 %; 14.6 %, 23.5 % and 31.5 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 5.8 %, 11.0 %, 35.5 %, 44.6 % and 60.9 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 13.7 %, 25.0 %; 45.3 %, 61.3 % and 62.8 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 19.9 %, 31.2 %; 49.4 %, 68.2 % and 74.3 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

For paraquat, at one hour (1 hr) period of observation, the rate of inhibitions were 2.7 %, 6.1 %; 7.8 %, 11.2 % and 16.7 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 8.7 %, 13.2 %, 23.5 %, 29.4 % and 39.0 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 13.1 %, 22.8 %; 31.1 %, 40.1 % and 48.4 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 25.9 %, 37.9 %; 47.9 %, 59.1 % and 70.0 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

Generally, for all the pesticide formulation used the rate of inhibitions were observed to increase as the concentration of the pesticide treatment increases. The lowest percentage of inhibition for *Nitrosomonas* sp. were observed in 0.5FR treated culture medium while the highest inhibitory rate was observed in 8FR treated culture medium throughout the period of observation. Likewise, the rate of inhibition was observed to increase with increase in the period of incubation.

Table 1: Percentageinhibitionof pesticides on *Nitrosomonas* sp.

Pesticide	**Percentage inhibition (%)**			
	Period (hour)			
	1	2	3	4
Concentrations				
0.5FR	3.1	9.5	25.8	37.0
FR	7.2	18.9	38.5	53.9
2FR	17.1	47.6	62.5	75.9
4FR	25.6	53.4	73.2	83.4

	8FR	32.4	73.0	78.9	90.0
	0.5FR	3.0	9.2	12.7	19.5
	FR	4.3	6.9	13.7	25.2
	2FR	10.6	20.8	40.6	54.1
Cypermethrin	4FR	20.9	41.6	64.8	72.4
	8FR	43.7	62.4	72.4	77.5
	0.5FR	1.5	5.8	13.7	19.9
	FR	5.3	11.0	25.0	31.2
	2FR	14.6	35.5	45.3	49.4
Glyphosate	4FR	23.5	44.6	61.3	68.2
	8FR	31.6	60.9	62.8	74.3
	0.5FR	2.7	8.7	13.1	25.9
	FR	6.1	13.2	22.8	37.9
	2FR	7.8	23.5	31.1	47.9
Paraquat	4FR	11.2	29.4	40.1	59.1
	8FR	16.7	39.0	48.4	70.0

The results on inhibitory effect of pesticide formulations were presented in Table 2. For endosulfan, at one hour (1 hr) period of observation, the rate of inhibitions were 1.3 %, 5.1 %; 8.1 %, 16.2 % and 24.6 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 5.6 %, 12.3 %; 19.9 %, 33.4 % and 53.3 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 8.4 %, 21.7 %; 33.8 %, 47.8 % and 60.2 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 23.5 %, 44.3 %; 51.1 %, 59.6 % and 70.9 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

For cypermethrin, at one hour (1 hr) period of observation, the rate of inhibitions were 3.0 %, 3.4 %; 12.2 %, 21.3 % and 25.3 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 5.0 %, 10.9 %, 18.5 %, 40.4 % and 63.9 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 14.0 %, 17.1 %; 35.5 %, 65.1 % and 71.0 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 25.4 %, 37.7 %; 58.0 %, 72.7 % and 75.8 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

For glyphosate, at one hour (1 hr) period of observation, the rate of inhibitions were 3.5 %, 5.8 %; 9.9 %, 17.0 % and 24.7 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 11.2 %, 13.0 %, 32.7 %, 36.7 % and 52.7 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 15.3 %, 35.1 %; 51.3 %, 59.6 % and 69.6 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 25.5 %, 41.7 %; 59.1 %, 70.3 % and 74.8 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

For paraquat, at one hour (1 hr) period of observation, the rate of inhibitions were 1.4 %, 4.7 %; 7.2 %, 8.6 % and 11.5 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 2 hr the rate of inhibition recorded were 6.5 %, 10.3 %, 13.7 %, 16.1 % and 20.2 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 3 hr period of observation, the rate of inhibitions were 18.3 %, 23.5 %; 28.7 %, 32.0 % and 38.1 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium. At 4 hr period of observation, the rate of inhibitions were 28.0 %, 38.1 %; 47.5 %, 54.5 % and 65.8 % respectively for 0.5FR, FR, 2FR, 4FR and 8FR treated culture medium.

Generally, for all the pesticide formulation used the rate of inhibitions were observed to increase as the concentration of the pesticide treatment increases. The lowest percentage of inhibition for *Nitrobacter* sp. were observed in 0.5FR treated culture medium while the highest inhibitory rate was observed in 8FR treated culture medium throughout the period of observation. Likewise, the rate of inhibition was observed to increase with increases in the period of incubation.

Table 2: Percentage inhibition of pesticide on *Nitrobacter* sp.

Pesticide		**Percentage inhibition (%)**			
		Period (hour)			
		1	2	3	4
Endosulfan					
	0.5FR	1.3	5.6	8.4	23.5
	FR	5.1	12.3	21.7	44.3
	2FR	8.1	19.9	33.8	51.1
	4FR	16.2	33.4	47.8	59.6
	8FR	24.6	53.3	60.2	70.9
Cypermethrin	0.5FR	3.0	5.0	14.0	25.4
	FR	3.4	10.9	17.1	37.7
	2FR	12.2	18.5	35.5	58.0
	4FR	21.3	40.4	65.1	72.7
	8FR	25.3	63.9	71.0	75.8
Glyphosate	0.5FR	3.5	11.2	15.3	25.5
	FR	5.8	13.0	35.1	41.7
	2FR	9.9	32.7	51.3	59.1
	4FR	17.0	36.7	59.6	70.3
	8FR	24.7	52.7	69.6	74.8
Paraquat	0.5FR	1.4	6.5	18.3	28.0
	FR	4.7	10.3	23.5	38.1
	2FR	7.2	13.7	28.7	47.5
	4FR	8.6	16.1	32.0	54.5
	8FR	11.5	20.2	38.1	65.8

The results for the median lethal concentration of the pesticide formulation at 4 hr exposure period were presented in Table 3. The median lethal concentration LC_{50} values for pesticides formulation evaluated for *Nitrosomonas* sp. in broth culture at 4 hr incubation period were 4.37μg / l, 12.73 μg / l, 14.62 μg / and 10.02 μg / l for endosulfan, cypermethrin, glyphosate and paraquat respectively (Table 3). The median lethal concentration LC_{50} value for pesticides formulation evaluated for *Nitrobacter* sp. in broth culture at 4 hr incubation period were 11.16 μg / l,10.21 μg / l, 10.50 μg / l and 11.15 μg / l for endosulfan, cypermethrin, glyphosate and paraquat respectively (Table 3).

Effects of pesticide on avoidance behaviour of *Ephyriodrilus* spp. in pesticide treated soils were presented in Table 4.

In endosulfan treated soils, the percentage avoidance response of *Ephyriodrilus* spp. were

Table 3: Median lethal concentration LC_{50} ($\mu g/l$) of pesticide formulations at 4 hr period of observation.

Pesticide formulations	LC_{50} ($\mu g/l$)	
	***Nitrosomonas* sp.**	***Nitrobacter* sp.**
Endosulfan	4.37	11.16
Cypermethrin	12.73	10.21
Glyphosate	14.62	10.50
Paraquat	10.02	11.15

Key: Median lethal concentration LC_{50} ($\mu g/l$) values were obtained from the logarithmic regression plots of percentage inhibition against the pesticide application rates.

11.0 %, 22.4 %, 44.4 %, 77.6 % and 88.8 % respectively in soil spiked with pesticide concentration of the normal application rate (0.5FR), the normal application rate (FR), two times the normal application rate (2FR), four times the normal application rate (4FR) and eight times the normal application rate (8FR) (Table 4).

In cypermethrin treated soils, the percentage avoidance responses of *Ephyriodrilus* spp. were 11.0 %, 33.4 %, 55.6 %, 88.8 % and 100.0 % respectively in soil spiked with 0.5FR, FR, 2FR, 4FR and 8FR pesticide concentrations (Table 4).

The percentage avoidance response of *Ephyriodrilus* spp. in glyphosate treated soils at 0.5FR, FR, 2FR, 4FR and 8FR pesticide concentration were 11.0 %, 22.4 %, 33.4 %, 55.6 % and 66.6 % respectively (Table 4).

In paraquat treated soil, the percentage avoidance response of *Ephyriodrilus* spp. at 0.5FR, FR, 2FR, 4FR and 8FR were 0.0 %, 11.0 %, 11.0 %, 33.4 % and 66.6 % respectively (Table 4).

The percentage distribution of *Ephyriodrilus* spp. in pesticide spiked soils were observed to be significantly reduced ($p < 0.05$) in the soils spiked with pesticides above recommended field rate (Table 4).

Less than 20 % *Ephyriodrilus* spp. were observed in the soil spiked with 4FR (11.2 %) and 8FR (5.6 %) endosulfan concentration; 4FR (5.6 %) and 8FR (0.0 %) cypermethrin concentration and 8FR (16.7 %) glyphosate and paraquat concentration respectively (Table 4).

Table 4: Effect of pesticide on the avoidance behaviour of earthworm.

Pesticide	Rate	Distributions (%)		Avoidance (%)
		Control	Treated	
Endosulfan	0.5FR	55.5	44.5	11.0
	FR	61.2*	38.8	22.4
	2FR	72.2*	27.8	44.4
	4FR	88.8*	11.2	77.6^{r}
	8FR	94.4*	5.6	88.8^{r}
Cypermethrin	0.5FR	55.5	44.5	11.0
	FR	66.7*	33.3	33.4
	2FR	77.8*	22.2	55.6
	4FR	94.4*	5.6	88.8^{r}
	8FR	100.0*	0.0	100^{r}
Glyphosate	0.5FR	55.5	44.5	11.0
	FR	61.2*	38.8	22.4
	2FR	66.7*	33.3	33.4

	4FR	77.8*	22.2	55.6
	8FR	83.3*	16.7	66.6^{r}
Paraquat	0.5FR	50.0	50.0	0.0
	FR	55.5	44.5	11.0
	2FR	55.5	44.5	11.0
	4FR	66.7*	33.3	33.4
	8FR	83.3*	16.7	66.6^{r}

Keys: * showed significant level in percentage distribution at $p = 0.05$; ^{r} indicates reduced habitat function (RHF) for percentage distribution that is < 20%.

Suitability of sentinel organisms for pollutants' ecotoxicology studies in the environment had been previously appraised by several researchers (Gouveia *et al*., 2018; Aborisade *et al*., 2021; Singh and Fatima, 2022). These sentinel species are found across all the hierarchical classes of living organisms and play important roles in ecosystem sustainability. The ecological significant microbial species of soil such as nitrifiers (*Nitrosomonas* sp. and *Nitrobacter* sp.) and macrofauna species such as earthworms were among the favoured tool for ecotoxicology studies (Atuanya and Tudararo-Aherobo, 2015; Atuanya *et al*., 2018; Singh and Fatima, 2022). Thus, the ecotoxicology studies of endosulfan, cypermethrin, glyphosate and paraquat pesticide formulations were accessed *in vitro* in Winogradsky media on *Nitrosomonas* sp. and *Nitrobacter* sp. and in pesticide spiked soil on *Ephyriodrilus* spp.

Generally, the pesticide formulations (endosulfan, cypermethrin, glyphosate and paraquat) were observed to exhibit higher inhibitory rate on *Nitrosomonas* sp. and *Nitrobacter* sp. as the concentration of the pesticide treatments increases. Also, the results showed that the inhibitory effect was also dependent on the period of exposure as the effect was more evident as the exposure period increases. Similar observations have been made by Ibiene and Okpokwasili (2011), who observed an increase in the toxicities of lindane, diazion and carbofuran formulations on *Nitrosomonas* and *Nitrobacter* sp. with increases in contact period and concentrations of the agro-insecticide.

The median lethal concentration (LC_{50}) values after 4 hours exposure period indicated toxicity level of the pesticide formulations to nitrifiers. Comparatively, the LC_{50} values of each pesticide on *Nitrosomonas* sp.and *Nitrobacter* sp. indicates that endosulfan and paraquat were more toxic to *Nitrosomonas* sp. in broth medium than *Nitrobacter* sp.; while cypermethrin and glyphosate are more toxic to *Nitrobacter* sp. than *Nitrosomonas* sp. after a 4hr exposure period. Furthermore, among the pesticide formulations, endosulfan followed by paraquat then cypermethrin were relatively more toxic to *Nitrosomonas* sp. than glyphosate. Cypermethrin, followed by glyphosate, then paraquat have more toxic effects on *Nitrobacter* sp. thanendosulfan. Takagi *et al*. (1994) observed that s-triazine pesticide caused more inhibitory effects on *Nitrosomonas* sp. compared to its effect on *Nitrobacter* sp. Ibiene and Okpokwasili (2011) also reported that lidane, diazon and cabofuran comparatively have different toxic effects on *Nitrosomonas* and *Nitrobacter* sp.

The differences in the toxic effects of these pesticides on *Nitrosomonas* and *Nitrobacter* sp. may be attributed to differences in chemical composition of the pesticide formulations and susceptibility potentials of the test organisms. Previous research has identified that the magnitude of toxicity of chemical formulation in the environment could be affected by the nature of chemical formulations, route of exposure, inherent physiological versatility and genetic make-up of the organism (Takagi *et al*., 1994). Also, the sudden exposure of *Nitrosomonas* and *Nitrobacter* sp. to the pesticide treatments in broth culture without any pre-adaptation or exposure phase may have contributed to the adverse effects of these pesticides. Busse *et al*.

(2000) reported that glyphosate pesticide was lethal to bacteria and fungi when applied in soil free media and observed a significant stimulation in microbial growth and activity when added directly to the soil. Furthermore, other studies have confirmed the sensitivity of *Nitrosomonas* and *Nitrobacter* sp. to chemical pollutants such as Azo dyes, drilling fluids and plastic (Ogugbue and Oranusi, 2005; Odokuma and Akponah, 2008; Atuanya *et al*., 2016). These organisms (*Nitrosomonas* and *Nitrobacter* sp.) are known to play major ecological roles in nutrient circulation through the process of nitrogen transformation in the ecosystem (Sudhakar *et al*., 2000). Therefore, any pollutant (including pesticide) that could have adverse effects on their growth may hinder the important ecological functions rendered by these organisms.

Earthworms are important components in the agro-ecological system due to their indispensable contributions to the maintenance of soil functions and fertility (Ibtissem *et al*., 2012). They are important tools in soils ecotoxicology studies because of their direct contact to soil, the presence of highly sensitive and receptive prostomium and anterior chemoreceptor tubercles and the sensory cells in their mouth region (Pereira *et al*., 2010; Singh and Fatima, 2022). Studies have confirmed the avoidance tests as an emerging technique in ecotoxicological screening that offer rapid, sensitive and inexpensive methods of assessing the impact of chemical pollutants on soil invertebrates (Latha and Basha, 2019; Gunstone *et al.,* 2021).

The results of the effect of pesticide (endosulfan, cypermethrin, glyphosate and paraquat) on avoidance behaviour of sentinel microfauna revealed that *Ephyriodrilus* spp. significantly avoided ($p < 0.05$) the soils treated with all the pesticide treated soil at the concentration above recommended field rate. On the other hand, more earthworms were observed in pesticide free soil of the experimental set-up. De-Silva and Amarasinghe (2008) reported that earthworm (*Eisenia andrei*) responded negatively to a tropical soil treated with different concentration (1-300 mg/kg) of dimethoate pesticide at the concentration above the recommended rate. Garcia *et al*. (2008) also observed a marked avoidance response of earthworms in tropical soil treated with carbendazim and lambda-cyhalothrin pesticide respectively at concentration ranging from 1 – 1000 mg/kg.

Sousa and Andrea (2011) noticed that the earthworms showed more preference to the untreated soil as compared to cypermethrin treated soil at 15, 30 and 60 μg/g application rate respectively. Furthermore, Alves *et al*. (2013), who studied the impact of insecticide (imidacloprid, fipronil and thiametoxam) and fungicide (captan and carboxi-thiram) on the avoidance behaviour of *Eisenia andrei* under tropical conditions reported that, while imidacloprid, captan, carboxi-thiram and thiametoxam treated soils were avoided by the *Eisenia andrei*; the earthworms rather showed a marked preference for soils treated with the fipronil insecticide.

According to International Organization for Standardization (ISO, 2007) as reported by De-Silva and Amarasinghe (2008) in an ecotoxicology assessment, a particular chemical pollutant could cause reduced habitat functions in the soil system 'if 80% or more of the test organism showed preference for the untreated soils' (i.e., ≤ 20% has preference for treated soil). Hence, the present results revealed less than 20 % *Ephyriodrilus* spp. in the soil spiked with 4FR (11.2 %) and 8FR (5.6 %) endosulfan concentration; 4FR (5.6 %) and 8FR (0.0 %) cypermethrin concentration and 8FR (16.7 %) glyphosate and paraquat concentration respectively. This is an indication that at this application rate, these pesticides could cause reduced habitat function to *Ephyriodrilus* spp.

Generally, an increase in concentration of pesticide treatment resulted in marked decrease in number of earthworms that avoided the treated soils and consequently resulted in significant differences ($p < 0.05$) in the distribution of earthworm between the untreated soils and the treated soil.

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