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Toxicity of Quaternary Mixture of Formulated Glyphosate and Phenols on *Providencia vermicola* Dehydrogenase Activity

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FNO and MUO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FNO, CON and BOU managed the analyses of the study. Authors FNO, SCO, BOU and CUD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the toxicity of glyphosate (as an active ingredient in drysate), three phenols (2,4-dichlorophenol, 4-chlorophenols and phenol) and quaternary mixtures of glyphosate, 2,4-dichlorophenol, 4-chlorophenol and phenol on the dehydrogenase activity (DHA) of *P. vermicola*.
Study Design: The glyphosate, 2,4-dichlorophenol, 4-chlorophenol and phenol mixture ratios (%) were designed as: 25%:25%:25%:25%; 30%:20%:20%:30%, 40%:10%:10%: 40%; 50%:5%:30%:15% and 20%:20%:10%:50% for the respective mixtures in the concentration range of 0-1000 mg/L.

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Place and Duration of Study: Silver Press Laboratory, Owerri Nigeria between July, 2016 and August, 2019.
Methodology:A laboratory scale study was carried on four toxicants using dehydrogenase inhibition test. The inhibition of dehydrogenase activity of the isolate by toxicant was calculated relative to the control. The percentage inhibition data generated was fitted into a logistic dose response model and Weibulleum to obtain their respective IC_{50}.
Results: The results revealed that the median inhibitory concentrations (IC_{50}) of the mixtures on the *Providencia vermicola* were 247.17 ±10.20, 163.44 ± 8.18, 251.33 ± 45.25, 267.30 ± 8.76 and 178.93 ± 45.53 mg/L, respectively. Toxicity index (TI) model was used to evaluate the joint action toxicity of the mixtures on the test organism and was 60 % antagonistic.
Conclusion: Thus, these synergistic and additive interactions of formulated glyphosate with the intermediates of 2,4-D was possible against the dehydrogenase activity of *Providenciavermicola* an important plant growth promoting bacterium. The dynamics of the toxic effects thus would depend on the concentration of the compounds.

Keywords: Dehydrogenase activity; glyphosate; herbicide; phenols; toxicity.

1. INTRODUCTION

Use of herbicides is increasing in the worldwide crop production [1]. Weed control using herbicides increases crop yield over manual weeding Hossain *et al.* [2]. Thus, herbicides are being rapidly adopted in developing countries because of shortages of hand weeding labour and the need to raise crop yields [3]. The use of herbicides has gained impetus from the general rise in farm wages. The belief that using herbicides is more beneficial for controlling weeds has led to the adoption of herbicides in the larger world for the upcoming developed agriculture.

One of the most commonly used herbicides is glyphosate [N-(phosphonomethyl)glycine], a post-emergence herbicide that inhibits the photosynthetic processes in plants. In order to improve the herbicidal action, glyphosate is often used in combination with other herbicides including 2,4-dichlorophenoxyacetic acid (2,4-D) [4]. In soils, these herbicides are liable to biodegradation by microorganisms and other biological factors. Glyphosate is moderately persistent in soil with half-life ranging from 2 to 197 days [5]. The intermediates of 2,4-D biodegradation and glyphosate could interact in the environment where mixture of the herbicides was applied. In Drysate one of its commercial formulation, glyphosate is formulated as isopropylamine (IPA) salt of glyphosate 360 g/l and a surfactant, polyoxyethyleneamine (POEA). When applied at recommended rates, glyphosate can be mineralized by soil microorganisms leading to increase in microbial biomass [6].

Phenol is an aromatic organic compound with the formula C_6H_50H. It is a white crystalline solid that is volatile. The molecules consist of a phenyl group (C_6H_5) bonded to a hydroxyl group (0H). Biological treatment of phenols has emerged as an increasingly important method in pollution management [7]. Chlorophenols (CPs) are aromatic ring structures containing at least one chlorine atom (−Cl) and one hydroxyl (−OH) group at the benzene rings. They are ubiquitous contaminants in the environment [8–10]. Their thermal, biological and chemical degradation is responsible for the release of harmful metabolites which constitute public health problems. They are also referred to as xenobiotic contaminants, which form a significant part of all organic chemicals either produced or used by many industries such as petrochemicals, oil refineries, plastics, insulation materials, pesticides, biocides, pulp and wood preservers [11]. Some particularly mono-chlorophenols can be formed during the breakdown of pesticides and other chlorinated aromatic compounds [12]. Due to their high toxicity, strong odor emission, persistence due to difficulty in cleaving of the benzene ring [13] and suspected health issues, phenolics pose critical ecological issues to the environment [11]. Most of the phenolis have been included in the US Environmental Protection Agency EPA list of priority pollutants [11, 14]. However, higher concentration levels of chlorophenols ranging from 0.15 to 200 mg/L were frequently found in contaminated environments [15]. However, several microorganisms can tolerate them for source of carbon and energy [16]. Furthermore, the recalcitrant nature of phenolic compounds to degradation is responsible for its persistence, and so a comprehensive understanding of the effect of their interactive toxicity with herbicides in the soil environment to the non-target soil microorganisms should be evaluated. To best of our knowledge, much work has not been done in

this regard. In this study, we evaluated the toxicity of glyphosate as active ingredient in drysate alone and in mixture with 2,4-dichlorophenol, 4-chlorophenol and phenol based on inhibition of dehydrogenase activity in *Providencia vermicola* an important plant growth rhizobacterium.

2. MATERIALS AND METHODS

2.1 Test Organism

The test organism, *Providenciavermicola* was isolated from the rhizosphere of *Oryzasativa* in its vegetative stage of growth. One gram of the rhizospheric soil sample free of debris was placed into sterile 3 ml distilled water. After serial dilution, a portion of 0.1 mL aliquots of 10^{-3} dilution factor was plated out using spread plate technique on nutrient agar medium that contained Nystatin (100 µl/mL) and incubated for 24 – 48 hr at room temperature (28 ^{0}C ± 2.00 ^{0}C). The culture was purified and characterized biochemically using standard microbiological methods. Pure cultures were stored on nutrient agar slants at 4°C [17].

2.2 Preparation of Inoculum for Toxicity Assay

A 24 hr culture of cell 10^6 CFU/g of the test organism grown in nutrient broth on a rotary shaker (150 rpm) at 28 ^{0}C ± 2.00 ^{0}C was harvested by centrifugation (3500 rpm, 10 min). The harvested cells were washed three times in sterile normal saline and suspended therein. Normal saline was used to zero the spec at 540 nm. The optical density (A_{540}) of the cell suspension was adjusted to 0.1 [17].

2.3 Quaternary Mixture Ratio

The quaternary mixtures consisted of glyphosate (as the active ingredient in formulated glyphosate herbicide, Drysate) and phenolic compounds (which includes 2,4-dichlorophenol, 4-chlorophenol and phenol). The quaternary mixtures studied were 25 %:25 %:25 %:25 %, 30 %:20 %:20 %:30 %, 40 %:10 %:10 %:40 %, 50 %:5 %:30 %:15 % and 20 %:20 %:10 %:50 % for the respective mixtures in the concentration range of 0-1000 mg/L [17].

2.4 Toxicity Assay using Inhibition of Total Dehydrogenase Enzyme Activity

The 2,3,5-triphenyltetrazolium chloride (TTC)-dehydrogenase activity assay was done in 2000 µL volume of nutrient broth (pH 7) which was supplemented with varying concentrations of glyphosate, 2,4-dichlorophenol, 4-chlorophenol and/or phenol. A 500 µL portion of nutrient broth and requisite volumes of sterile water and stock solutions (0 – 2000 mg/L) of respective glyphosate and/or phenolic compound were added to each tube to obtain the different quaternary mixtures ratios. After which 100 µL bacterial suspension was added and incubated for 24 hr. A of 100 µL of 0.1 % aqueous solution of TTC was then added into each tube. The final concentrations of the toxicants ranged from 0 to 1000 mg/L. The controls consisted of parameters other than phenols or glyphosate. They were incubated at 28 ^{0}C ± 2.00 ^{0}C for 24 hr. After which the TTC formazan produced was extracted in 4000 µL butanol. The optical density of the extract was determined spectrophotometrically at 540 $_{nm}$ (visible spectrophotometer 72UD by life Science Instrument Company). The inhibition of dehydrogenase activity of the isolate by toxicant was calculated relative to the control. The % inhibition for organisms was plotted against the concentration of the toxicants or mixtures using statistical software (Table Curve 2D v5.01, Systat, USA). This was fitted into appropriate statistical models and the toxicity threshold concentration (IC) of the extracts against the isolate evaluated using the software [17].

2.5 Data Analysis

2.5.1 Determination of toxicity thresholds

The percentage inhibition of dehydrogenase activity of the isolates induced by the individual component and that of the quaternary mixtures of glyphoaste, 2,4-dichlorophenol, 4 –chlorophenol and phenol were calculated relative to control using equation 1. The percentage inhibition data generated was fitted into logistic dose response model (LDR (a,b,c) (eqn 2), LDR (a,b,c,d) (eqn 3). The dose-response data were fitted to obtain their respective IC_{50} which is the concentrations of the toxicants that inhibited 50% of dehydrogenase activity of the bacterial. All curve fittings were done using Sigmaplot 10 and Table Curve 2D v5.01. Duncan test was used for the statistical analysis [17].

$$\%\ Inhibition = \left[\frac{Control_{ABS} - Test_{ABS}}{Control_{ABS}}\right] \times 100 \quad (1)$$

Logistic dose response model

$$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c} \quad (2)$$

Where: x is the concentration of the toxicant, a is the maximum response (of untreated control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}.

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d} \quad 2$$

Where: x is the concentration of the toxicant, b is the maximum response (of untreated control), c is the IC_{50}, d is parameter determining the relative slope at IC_{50}.

$$y = a\left[1 - exp\left[-\left[\frac{x + c(In\ 2)^{1/d} - b}{c}\right]^d\right]\right] \quad 3$$

Where: x is the concentration of the toxicant, a is the maximum response (of untreated control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}.

2.5.2 Determination of toxic unit (TU)

The toxicities of the mixture components expressed in TU for a given ICp were calculated using Weibullcum model (a,b,c,d) (eqn 4), from equations 4 and 5.

$$TU_A = \frac{C_{mixA}}{IC_{pA}} \quad 4$$

$$TU_B = \frac{C_{mixB}}{IC_{pB}} 4$$

Where TU_A , TU_B, TU_C and TU_D are the toxicity unit of components A, B,C and D of the mixtures respectively, ICpA, ICpB, ICpC and ICpD are the toxicities (ICp) of components A, B, C or D respectively determined individually, and CmixA, CmixB, CmixC and CmixD are the concentrations of component A, B, C and D at ICp of the mixture. CmixA, CmixB, CmixC and CmixD can be calculated by multiplying the ratio of individual components in the mixture by the ICp of the mixture [ICpmix (A,B,C,D)] as follows:

$$C_{mixA} = \frac{A\%}{100} \times IC_{pmix(A,B,C,D).} \quad 5$$

$$C_{mixB} = \frac{B\%}{100} \times IC_{pmix(A,B,C,D)} \quad 5$$

$$C_{mixB} = \frac{C\%}{100} \times IC_{pmix(A,B,C,D)} \quad 5$$

$$C_{mixB} = \frac{D\%}{100} \times IC_{pmix(A,B,C,D)} \quad 5$$

Where A %, B%, C% and D % are the relative amount of components A or B or C or D respectively in the mixture (A %, B%, C% and D % ≠ 0). When A = 0 %, CmixA = 0 and CmixA = ICpA, When B = 0 %, CmixB = 0 and CmixB = ICpB, When C = 0 %, CmixC = 0 and CmixC = ICpC and When D = 0%, DmixD = 0 and CmixB = ICpD.

2.5.3 Analysis of combined effects using toxic index model

Toxic index (TI) model was used to analyze the combined effect of the quaternary mixtures. The TI values were calculated as follows (equation 6).

$$TI = \frac{C_{mixA}}{IC_{50A}} + \frac{C_{mixB}}{IC_{50B}} + \frac{C_{mixC}}{IC_{50C}} + \frac{C_{mixD}}{IC_{50D}} 6$$

Where CmixA, CmixB, CmixC and CmixD are the concentrations of components A, B, C and D respectively at the IC_{50} of the mixture; IC_{50}A, IC_{50}B, IC_{50}C and IC_{50}D are the IC_{50} of component A, B, C and D respectively, measured individually.

TI = 1 describes additive interaction, TI > 1 describes antagonistic interaction and TI < 1 describes synergistic interaction [18].

3. RESULTS

3.1 Morphological and Biochemical Characteristics of the Test Organism

Colonies on nutrient agar medium were luxuriantly growing brownish colonies with coccobacillus shape after 24 hr incubation at room temperature (28^0C + 2.00^0C). Microscopic test revealed the organism as Gram negative, motile rods. The biochemical characteristics are given in the Table 1 below:

3.2 Toxicity of Single Chemicals

The response of the dehydrogenase activities (DHA) of *Providenciavermicola* to the stress of g;yphosate, 2,4-dichlorophenol.4-chlorophenol and phenol as individual substance and the mixtures showed that the substances have biphasic effect on the enzyme activity (Figure 1). There was DHA stimulation (hormesis) at low doses and inhibition at high doses. With the exception of 4-chlorophenol that progressively

Table 1. Morphological and biochemical characteristics of the isolate

Property	Test organism
Gram reaction and shape	Gram negative rod
Spore	-
Motility	+
Catalase	+
Indole test	+
Oxidase test	-
Urease test	+
NO_3 reduction test	+
H_2S test	-
Citrate utiliation test	+
Identity	*Providenciavermicola*

= Negative result, + = Positive result, NO_3 = Nitrate; H_2S = Hydrogen Sulphide

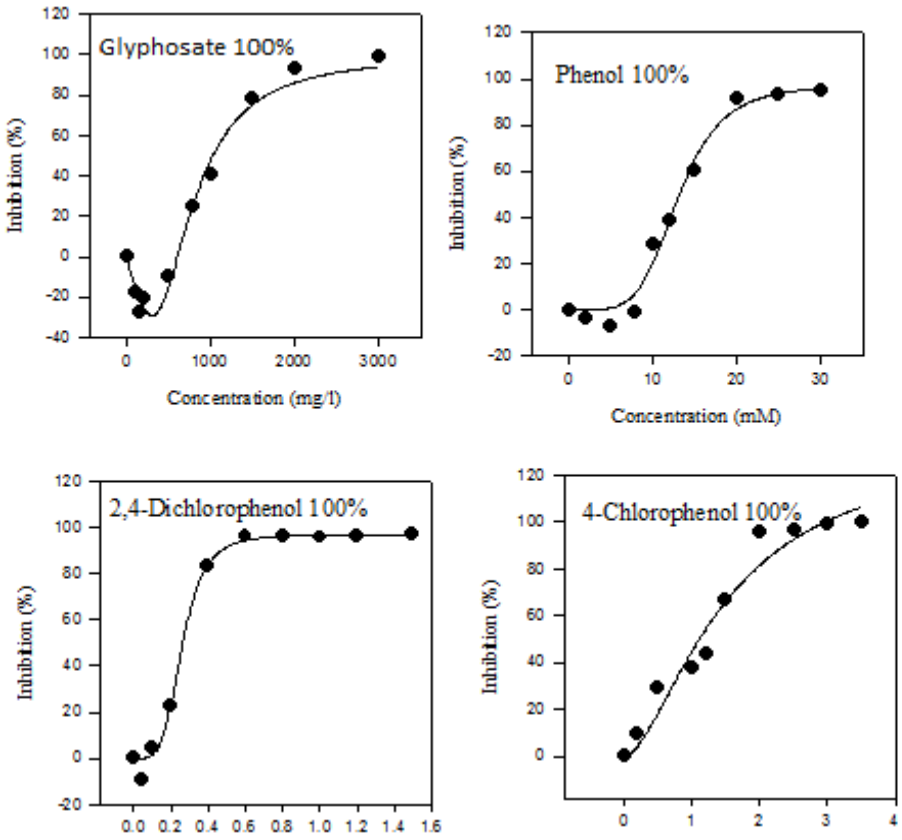

Fig. 1. Inhibitory response of *P. vermicola* dehydrogenase activity by phenol, glyphosate, 2,4-dichlorophenol and 4-chlorophenol

inhibited DHA of the test organism reaching saturation at 3.5 mM concentration, glyphosate, 2,4-dichlorophenol, and phenol as single substances stimulated the enzyme activity at low concentrations of up to 200 mg/L, 0.1 mM and 10 mM concentrations, respectively. At concentrations above the hormetic range, glyphosate, 2,4-dichlorophenol and phenol progressively inhibited DHA of the test organism reaching saturations at 3000 mg/L for glyphosate, 1.5 mM for 2,4-dichlorophenol and 30 mM concentrations for phenol. The decreasing order of toxicity was 2,4-dichorophenol > 4-chlorophenol >glyphosate> phenol.

As mixtures, the ratio 25 %:2 %:25 %:25 % and 40 %:10 %:10 %:40 % of glyphosate, 2,4-dichlorophenol, 4-chlorophenol and phenol stimulated DHA of the organism at concentrations ranging from 0-100 mg/L The above mixtures progressively inhibited DHA at concentrations above the hormetic range reaching saturation point at concentration between 800 mg/L and 1000 mg/L. Other quaternary mixture ratios evaluated exhibited progressive inhibition of the dehydrogenase activity of the bacterial isolate as the concentrations increased with total inhibition at 1000 mg/L concentration (Fig. 2).

The 24 hr toxicity thresholds (IC_{50}) of the substances are shown in Table 2. As single compounds 2,4-dichlorophenol with IC_{50} of 42.37 ± 1.56 mg/L was the most toxic chemical while phenol with IC_{50} of 1252.33 ± 16.62was the least toxic. However, there was a significant difference between the toxicity of glyphosate and that of the phenolics ($p < 0.05$). All the same, there was no significant difference between the toxicity of 2,4-dichlorophenol and 4-chlorophenol to the tested isolate ($p > 0.05$). As individual compounds, the increasing order of toxicity was 2,4-dichlorophenol > 4-chlorophenol > glyphosate > phenol.

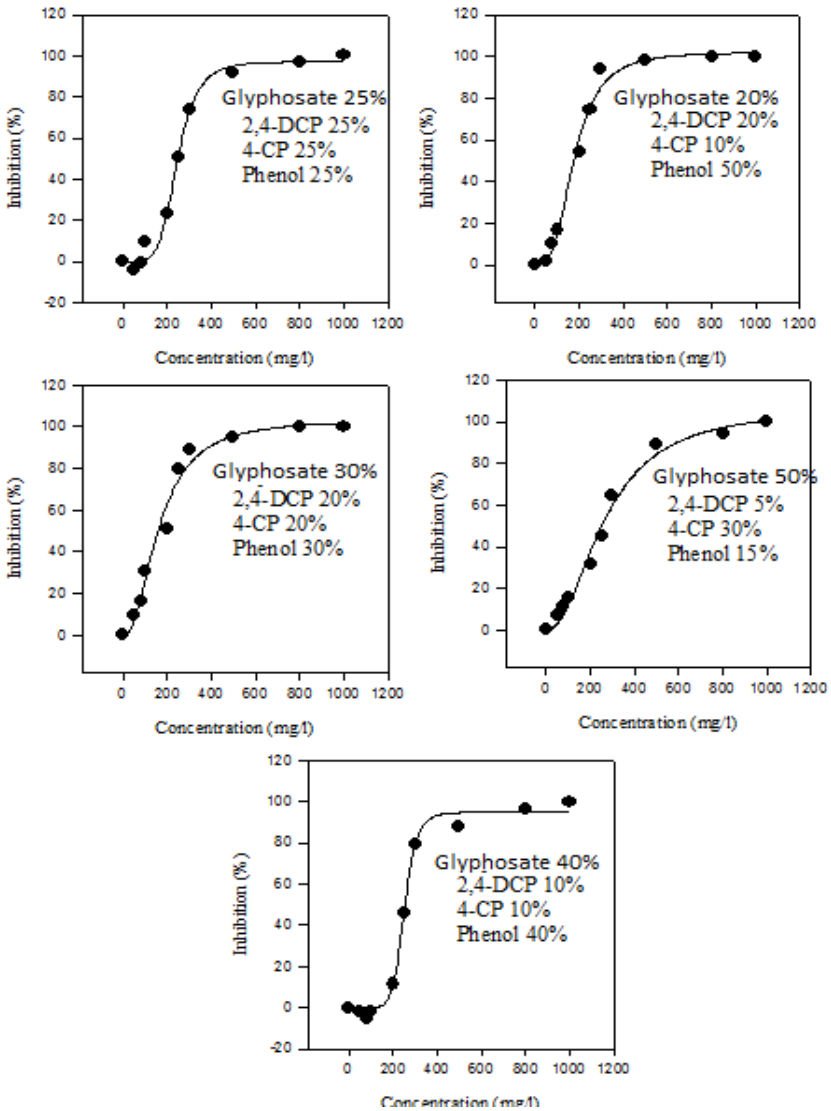

Fig. 2. Inhibitory response of *P. vermicola* dehydrogenase activity by quaternary mixtures of glyphosate, 2,4-dichlorophenol, 4-chlorophenol and phenol

Table 2. Inhibitory concentrations (IC_{50}) of the individual chemicals to dehydrogenase activities of *P. vermicola*

Toxicant	Toxicity threshold (IC_{50}) of *P. vermicola* (mg/L)
Glyphosate	366.18 ± 21.94[b]
2,4-Dichlorophenol	42.37 ± 1.56[a]
4-Chlorophenol	187.56 ± 8.98[a]
Phenol	1252.33 ± 16.62[c]

In each column, values with same letters are not significantly (p > 0.05) different

The 24 hr IC_{50} values of 163.44 ± 8.18 mg/l of 30 % + 20 % + 20 % + 30 % (glyphosate, 2,4-dichlorophenol, 4-chlorophenol and phenol) mixtures decreased with increase in percentage of 2,4-dichlorophenol (the highest toxic component). All the quaternary mixture ratio had IC_{50} values that were higher than the value (42.37 ± 1.56 mg/L) observed for the 2,4-dichlorophenol alone. In the case of 50 % + 5 % + 30 % + 15 % mixture with IC_{50} of 267.30 ± 8.76 mg/L, the IC_{50} values increased with increase in percentage of glyphosate (a less toxic component). The mixture ratio of 40 % + 10 % + 10 % + 40 % with IC_{50} value of 251.33 ± 45.25 mg/L increased with the increase in both glyphosate and phenol (the two least toxic components). The IC_{50} value 178.93 ± 45.53 mg/L of 20 % + 20 % + 10 % + 50 % mixture decreased by the increase in 2,4-dicholorophenol component (a highly toxic component). However, the effect of the mixture ratios of 25 % + 25 % + 25 % + 25 %, 40 % + 10 % + 10 % + 40 % and 50 % + 5 % + 30 % + 15 % on the test organism are not significantly different (p > 0.05). The effect of the mixture ratios of 30 % + 20 % + 20 % + 30 % and 20 % + 20 % + 10 % + 50 % are also not significantly different (p > 0.05). There was a significant difference between the most toxic mixture and the least toxic mixture (p < 0.05).

3.3 Toxic Index (TI) Analysis

According to the TI model, synergistic, additive and antagonistic effects were observed in the quaternary mixtures as presented in Table 4. With the exception of mixture ratios of 40 %:10 %:10 %:40 % that exhibited additive effect on DHA of the test organism and that of 20 %:20 %:10 %:50 % mixture ratio that revealed synergistic effect, antagonistic effect was observed in the other quaternary mixtures evaluated. 20 % additive, 20 % synergistic and 60 % antagonistic effect on the DHA of the test organism was observed in the quaternary mixtures evaluated.

Table 3. Inhibitory concentrations (IC_{50}) of the quaternary combinations of the chemicals to dehydrogenase activities of *P. vermicola*

Toxicant mixtures	Toxicity threshold (IC_{50}) mg/L *P. vermicola*
Quaternary25 % + 25 % + 25 % + 25 %	247.17 ± 10.20b
30 % + 20 % + 20 % + 30 %	163.44 ± 8.18a
40 % + 10 % + 10 % + 40 %	251.33 ± 45.25b
50 % + 5 % + 30 % + 15 %	267.30 ± 8.76b
20 % + 20 % + 10 % + 50 %	178.93 ± 45.53a

In each mixture, values with same letters are not significantly (p > 0.05) different

Table 4. Toxic index of the test chemicals in quaternary mixtures and their respective interactions on dehydrogenase activity of the bacteria *P. vermicola* according to T1 Model

Toxicant mixture	*P.vermicola*	
Quaternary mixtures	TI	Effect
Glyphosate: 2,4- dichlorophenol: 4-chlorophenol: Phenol		
25 %:25 %:25 %:25 %	1.980	Antagonistic
30 %:20 %:20 %:30 %	1.115	Antagonistic
40 %:10 %:10 %:40 %	1.078	Additive
50 %:5 %:30 %:15 %	1.129	Antagonistic
20 %:20 %:10 %:50 %	0.840	Synergistic

4. DISCUSSION

Herbicides and phenols in the environment adversely affect the activities of the microorganisms like the *P. vermicola.* Toxicity of glyphosate to bacteria and other microorganisms have been reported by Nweke *et al.* [18]. *Providencia vermicola* responds to the stresses of glyphosate, 2,4-dichlorophenol, 4-chlorophenol and phenol as single pollutants. The 2,4-dichlorophenol had a biphasic effect on the DHA oforganism reaching saturation at concentration of 0.15 mM. Glyphosate and phenol also presented hormetic effect on the DHA of the test organism. This agreed with the findings of Nweke *et al.* [18] who reported hormetic effects of glyphosate at low doses on the DHA of pure culture of *Rhizobium* species. Stimulation of dehydrogenase activity at low doses of glyphosate observed in this study disagreed with the findings of Busse *et al.* [19] who reported that glyphosate exhibits higher toxicity in soil-free media. However, several bacterial species have been demonstrated to grow on glyphosate and its biodegradation intermediate [20,21]. The observed hormesis could be due to increase in respiration at low concentrations of herbicide. Similarly, phenol encouraged the enzyme activity of the organism up to 9 mM) [22] with total inhibition at 30 mM concentration. This finding that phenol is stimulatory to microbial activity at low dose disagreed with the work of Boszezyk-Maleszak *et al.* [23] who established that phenol can be toxic even at a low dose. The 4-chlorophenol did not present any hormetic effect on the DHA of *P.vermicola* rather it progressively inhibited the activity of isolate with total inhibition at 3.5 mM concentration. This may be because chlorophenols are resistant to biodegradation as the enzyme activity needed for ring cleavage could be suppressed by the chloride atoms in the structure as stated by Olaniran and Igbinosa [24]. Phenolic compounds are known to disrupt membrane functions by causing loss of cytoplasmic membrane integrity. Dehydrogenase enzymes are membrane-associated; thus loss of membrane integrity will eventually affect their activity. The order of toxicity of single compounds 2,4-dichlorophenol > 4-chlorophenol > phenol > glyphosate obtained with pure culture of *Rhizobium* species reported by Nweke *et al.* [18] is similar to that obtained in this study 2,4-dichlorophenol > 4-chlorophenol > glyphosate > phenol. From the statistical analysis, it can be deduced that the toxicity of 2,4-dichlorophenol is not significantly different from that of 4-chlorophenol. The high toxicity of chlorophenols observed in this work agreed with the findings of Arora and Bae [25] who reported that the compounds are highly recalcitrant and harmful to all forms of life.

Most information and evidence of toxicity are those obtained from single compounds. Refining our understanding of mixture interactions can lead to better informed environmental management and decision making. In addition, exploring mixtures interactions can elucidate the mechanisms of action for specific toxicants which in many cases, are poorly understood. Toxicity of mixtures of compounds is therefore important to toxicology. However, at the quaternary mixture of glyphosate /2,4-dichlorophenol / 4-chlorophenol / phenol in the ratio of 40 %, 10 %, 10 %, 40 %, there was hormetic response by *P. vermicola* up to 100 mg/L but other combination mixture ratios evaluated showed progressive dose inhibition against DHA of the test organism with total inhibition at the same concentration of 1000 mg/L. This could be possible because of the competence of the *P. vermicola* in breaking down herbicides and using the substrates as source of carbon or nitrogen for its life activities. Hayat [26] stated that the health and fertility of soil depends on the competence of the soil microorganism present. The inhibitory concentrations (IC_{50}) for *P. vermicola* at the value of 42.37 ± 1.56 mg/L portrays very high toxicity, the lesser the IC_{50} the more toxic the compound. This corresponds to the toxicity trend of the chemicals of this study where the most toxic was 2,4-dichlorophenol. According to Boyd *et al.* [27], the toxicity of phenolic compounds will also increase according to the number of chlorines being substituted in phenol. The finding in this work agreed with the above statement, the 2,4-dichlorophenol with two chlorine atoms was found to be more toxic than the compound 4-chlorophenol with one chlorine atom attached. The inhibition concentration of quaternary mixture 30 % / 20 % / 20 % / 30 % with the IC_{50} value of 163.44 ± 8.18 mg/L has the highest toxicity threshold on *P. vermicola* and it could be because of the presence of 2,4-dichlorophenol and 4 - chlorophenol in the mixture that has enhanced the toxicity. Combination ratio of 50 % / 5 % / 30 % / 15 % with IC_{50} value of 267.30 ± 8.76 mg/L has the least toxicity. The toxicity of the mixture, probably, was modulated by the presence of glyphosate in it. The inhibition of DHA observed in this study at higher concentrations of the herbicides corroborated with other reports. An IC_{50} of 18.2 mg/L glyphosate for *Vibrio*

*fischeri*bioluminescence was reported by Nweke *et al.* [18]. He also reported IC_{50} of 162.0 mg/L and 24.9 mg/L for glyphosate (isopropylamine salt) and Roundup®, respectively.

The TU values as well as the TI model used to analyze mixture toxicity indicated similar results regarding toxicity of phenols and glyphosate mixtures against DHA in *P. vermicola*. Although, there was seemingly additive and synergistic responses to the joint action of the mixture, the TI value was 60 % antagonistic within the intervals of 1.129 – 1.980. The toxicity of the mixture was therefore proposed to be antagonistic.

5. CONCLUSION

The results revealed that the toxicity of the toxicants over 24 hr period can be ranked as 2,4-dichlorophenol > 4-chlorophenol > glyphosate > phenol. The analysis indicated possibility of additive, synergistic and antagonistic action depending on the relative ratios of the individual components. However, the TI model led to the conclusion that the joint action of the mixtures on *P. vermicola* DHA is antagonistic. This information is essential towards assessing the environmental risks. Natural processes that modulate the residual amounts of these herbicides in the environmental media play an important role in the overall response to the toxicity of these chemicals. To enlarge the conclusion of this study, joint action of these toxicants on microbial community of soil and aquatic environment is also required.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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