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In vitro Interactive Toxicity of Binary Mixtures of Selected Herbicides on *Lysinibacillusfusiformis*

Franca Ngozi Okafor[1*], Michael Uchenna Orji[1], Samuel Chinedu Onuorah[1], Bright Obidinma Uba[2], Chinweike Unoma Dokubo[2] and Joypat Ogechukwu Ofunnwa[2]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria.
[2]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, P.M.B. 02 Uli, Anambra State, Nigeria.

Authors' contributions

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

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ABSTRACT

Aims: To assess the toxicities of some herbicides as individuals and in binary mixtures to *Lysinibacillus fusiformis* isolated from *Oryzasativa* plant using dehydrogenase activity as an endpoint.
Study Design: The binary mixture consists of combination of any two herbicides selected from the three herbicides (drysate, weedcut and aminoforce) for the study The binary mixture ratios (%) were designed as: 50%:50%; 80%:20% and 20%:80% for the respective mixtures in the concentration range of 0 -10, 000 mg/L.
Place and Duration of Study: Silver Press Laboratory, Owerri Nigeria between July, 2016 and August, 2019.
Methodology: A laboratory scale study was carried on three toxicants using dehydrogenase inhibition test. The inhibition of dehydrogenase activity of the isolate by toxicant was calculated relative to the control. All the dose-response relationships of the individual toxicants and that of themixtures were described by logistic dose model and Weibullcum model parameter.

**Corresponding author: E-mail: ezenekwenf@yahoo.com;*

Results: The results revealed that the median inhibitory concentrations (IC_{50}) observed were 1,067.33 ± 36.68 mg/L for drysate; 2,180.00 ± 147.31 mg/L for weedcut and 4,550.00 ± 62.45 mg/L for aminoforce. Duncan tests indicated that the IC_{50} of the toxicants were significantly different from each other. Among the individual toxicants, the ascending toxicities ranking were aminoforce > weedcut > drysate. The responses of the test organism to the stresses of the toxicants were dose-dependent and the toxicants also progressively repressed the dehydrogenase activity as the concentration increased. All binary mixtures were strongly synergistic against the organism.
Conclusion: Thus, the toxicity of individual compound and synergistic effects of the mixtures of the toxicants indicates potential deleterious effects of both the individual chemicals and their mixtures to the rhizobacteria of *Oryza sativa* plant.

Keywords: Drysate; weedcut; aminoforce; herbicides; dehydrogenase activity; toxicity.

1. INTRODUCTION

Herbicides are being rapidly adopted in developing countries to clear weeds because of shortages of hand weeding labour [1] and this attitude has deleterious effects on beneficial soil microorganisms. Research has shown that, if enough hand weeding is done at the optimal times, crop yields will not be reduced by weed competition [2].The belief that using herbicides is more economical less time consuming for controlling weeds has led to its increased use in the larger world for the upcoming developed agriculture. If microorganisms are sensitive to a particular herbicide, its application will interfere with the vital metabolic activities of the microbes[3].

To date, scientific studies about the impact of glyphosate (the active ingredient in drysate) on soil microorganisms have provided contrasting results. Glyphosate increases soil microbial activity when the herbicide is added because microbes break it down and use it as a source of carbon, nitrogen or phosphorus [4] or it does not cause any effect [5], or leads to a substantial decrease in the abundance of soil microorganisms [6]. Application of glyphosate to our farms has affected farm yield adversely by decreasing the populations of microorganisms that suppress the disease-causing fungi in the farm [7]. This condition could change the balance of bacteria and fungi, in turn altering soil ecosystem functions and plant health [8]. Glyphosate interferes with the uptake of essential minerals in agricultural crops too [8].

Paraquat (the active ingredient in weed cut) is toxic to soil fungi and bacteria causing a reduction in some populations [9,10] like the beneficial nitrogen-fixing blue-green alga *Cylindrospermum* sp. found in rice paddy [11].

The active ingredient in herbicide-amino force is 2,4-dichlorophenoxyacetic acid. The metabolites of its biodegradation in laboratory experiments are detected as 1,2,4-benzenetriol, 2,4-dichlorophenol (2,4-DCP), 2,4-dichloroanisole (2,4-DCA), 4-chlorophenol (4CP), chlorohydroquinone (CHQ), volatile organics, bound residues, and carbon (iv) oxide [12]. In order to enhance herbicidal action, glyphosate is often used in combination with other herbicides like the aminoforce which contains 2,4-D as the active ingredient [13,14].

Herbicides may be directly or indirectly toxic to the soil microorganisms. Their interaction with other pollutants in the environment may result to increased or decreased deleterious effects in the environment and most especially to the living components of the ecosystem. There are limited literatures on binary toxicity mixtures of drysate, weedcut and aminoforce herbicides on rhizobacteria as most documented literatures are on single compound effect thereby limiting issues of environmental management and decision making. The available data on the internet are deficient in binary toxicity mixtures on *Lysinibacillus fusiformis* test organism and hence necessitate this study. The purpose of this study is to assess the toxicities of some herbicides as individuals and in binary mixtures to *Lysinibacillus fusiformis* isolated from *Oryzasativa* plant using dehydrogenase activity as an endpoint.

2. MATERIALS AND METHODS

2.1 Isolation of the Test Organism

The test organism *Lysinibacillus fusiformis* was isolated from the root nodules of mature *Oryza sativa* plant. The root nodules were washed thoroughly with sterile distilled water and surface

disinfected in sterile culture tubes with 75% ethanol for 5 minutes. After, the nodules were crushed with sterile glass rod. A portion of 3 ml of sterile normal saline was added to 1 g of crushed root tissue. After serial dilution, a portion of 0.1 mL aliquots of 10^{-3} dilution factor was plated out using spread plate technique on the surfaces of the solidified Nutrient agar (NA) media that contained Nystatin (100 µl/mL) in triplicates with the aid of a sterile glass spreader. They were incubated for 24 – 48 hrs at 28 ± 2.00 ^{0}C. The pure culture was characterized biochemically using standard microbiological methods and identified using Bergey's Manual of Determinative Bacteriology [14].

2.2 Culturing of The Test Organism

Lysinibacillusfusiformis was grown in nutrient broth (Peptone 5.0, NaCl 5.0, Beef Extract 1.5 and yeast extract 1.5) g/L pH 7.4 for 24 hr at 26 ± 2.00^{0}C on a rotary shaker at 200 rpm to the log phase of the bacteria growth. It was harvested by centrifugation (3500 rpm, 10min). Harvested cells were washed thrice in sterile normal saline and suspended therein. The optical density (OD) at λmax = 540_{nm} was adjusted to 0.1 [14].

2.3 Binary Mixture Ratios for the Toxicity Assay

The binary mixture consists of combination of any two herbicides selected from the three herbicides (drysate, weedcut and aminoforce) for the study. The percentage ratios of the mixtures are shown in Table 1. The mixtures were composed by preparing the stock solutions to the same concentrations, and required volumes by percentage of stock solution of each compound taken from the stock and mixed thoroughly to give a specific concentration mixture ratio. Each mixture was treated as a solution of a single toxicant during toxicity testing.

2.4 Dehydrogenase Activity Assay

Dehydrogenase activity was determined using 2,3,5-triphenyltetrazolium chloride (TTC) as the artificial electron acceptor. The assay was done in 2-mL volume of nutrient broth-TTC medium (pH 7.0) supplemented with varying concentrations of the toxicants in separate 20-mL screw-capped culture tubes. A 0.5 mL portion of x4-strenght nutrient broth and required volumes of sterile distilled deionized water and stock solutions (0 - 5000 mg/L) of a particular combined toxicant was added into a culture tube in triplicates to obtain varying total concentrations of the toxicant in each mixture ratio. A portion of 0.1 mL of the bacterial suspension was added and pre-incubated at 26 ± 2.00 ^{0}C for 24 hr. Thereafter, 0.1 ml of 0.1% aqueous solutions of TTC was added into each tube. The final total concentrations of the toxicants ranged from (20 to 600 mg/L). The controls consisted of the medium without toxicant. The tubes were incubated at 26 ± 2.00 ^{0}C for 24 hr. Afterwards, 4 mL butanol was added into each tube and agitated mechanically for 1 min to extract the red TTC-formazan produced by enzymatic reduction of TTC. Spectrophotometer (VIS Spectrophotometer 721D) at 500_{nm} was used to read the absorbance of each extract. The percentage Inhibition of dehydrogenase activity of the isolate by single and mixtures of toxicant was calculated relative to the control and was plotted against the concentration of the toxicants or mixtures were determined [14].

2.5 Data Analysis

Equation 1 below was used for calculating the percentage inhibition of DHA of the test organism relative to control. The data generated was fitted into logistic dose response model (LDR (a,b,c) (eqn 2), LDR (a,b,c,d) (eqn 3). Dose-response data were fitted into the logistic model in order to obtain their respective IC_{50}, Sigmaplot 10 and Table Curve 2D v5.01 were used for all curve fittings [14]. Duncan test was used for the statistical analysis.

$$\%\ \text{Inhibition} = \left[\frac{\text{Control}_{\text{ABS}} - \text{Test}_{\text{ABS}}}{\text{Control}_{\text{ABS}}}\right] \times 100 \qquad (1)$$

Logistic dose response model

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

Table 1. Binary combinations of toxicants for dehydrogenase activities of *L. fusiformis*

Drysate + Aminoforce	Drusate + Weedcut	Aminoforce + Weedcut
50%:50%	50%:50%	50%:50%
80%:20%	80%:20%	80%:20%
20%:80%	20%:80%	20%:80%

Where: x is the toxicant concentration, a is the highest response (of untreated control), b is the IC_{50}, c is the 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}.

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

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

2.5.1 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) from equations 4 and 5.

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

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

Where TUA, and TUB are the toxicity unit of components A and B of the mixtures respectively, *ICpA*, and *ICpB* are the toxicities (*ICp*) of components A or B respectively determined individually, and CmixA, and CmixB are the concentrations of component A and B at *ICp* of the mixture. CmixA and CmixB can be calculated by multiplying the ratio of individual components in the mixture by the *ICp* of the mixture [*ICpmix* (A, B)] as follows:

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

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

Where A% and B% are the relative amount of components A or B or C respectively in the mixture (A% and B% 0). When A = 0%, CmixA = 0 and CmixA = *ICpA*; When B = 0%, and CmixB = 0 and CmixB = ICpB.

2.5.2 Isobolographic analysis of the mixture toxicities

The estimated$IC_{50}$and TU values of the binary mixtures were plotted in an isobologram [15], a method by which the interactions of 2-substance mixtures A" and B" could be represented in two dimensions. The curve is a straight line that joins y and x axis and is referred to as the additivity line. However, if the effect is synergistic, the isobole of the AB mixture is located below the additivity isobole (lower left); whereas, if the effect is antagonistic, the isobole of the mixture will be located above the additivity line[16].

2.5.3 Analysis of combined effects using toxic index model

Toxic index (TI) model was also used to evaluate the combined effect of the binary mixtures. The values were calculated as follows (equation 6):

$$TI = \frac{C_{mixA}}{IC_{50A}} + \frac{C_{mixB}}{IC_{50B}} \quad (6)$$

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

TI=1, indicates additive interaction, TI > 1 indicates antagonistic and TI < 1 indicates synergistic interaction [15].

3. RESULTS

3.1 Phenotypical Identification of the Test Organism

3.2 Toxicity of the Individual Toxicants

The response of the test organism to the toxic effect of the herbicides was dose-dependent. The inhibition of *L. fusiformis* DHAbydrysate, weedcut and aminoforce as single compounds as well as the fit of the logistic and hormesis models are shown in Fig 1A-C. Weedcut presented hormetic response to the enzyme activity but repressed it as the concentration increased above the hermetic dose. Other herbicides progressively inhibited the enzyme activity. The decreasing order of toxicity was drysate > weedcut > aminoforce.

Table 2. Morphological, microscopic and biochemical features of the isolate

Property	Observation/Reaction
Colony colour	White
Shape	Rod
Gram reaction	+
Spore	+
Catalase	+
Indole test	+
Oxidase test	+
Urease test	+
Motility	+
Sugar fermentation	+
NO_3 reduction test	+
H_2S test	-
Citrate utiliation test	+
Identity	*Lysinibacillus fusiformis*

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

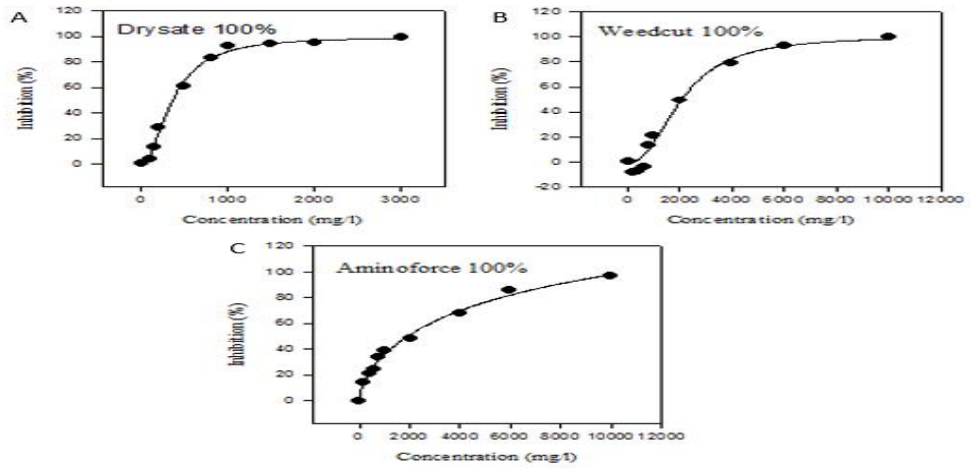

Fig. 1A-C. Inhibitory response of *L. fusiformis* dehydrogenase activity by Drysate, Weedcut and Aminoforce

3.3 Toxicity of Binary Mixtures of the Chemicals

The toxicity of binary mixtures of the chemicals are shown in Figs. 2 - 4. The results showed that the mixtures progressively repressed the enzyme activity of the isolate as the mixture concentration increased characterized by similar sigmoid curve. Binary combination of drysate + weedcut at concentration ratios of 50%:50% and 20%:80% showed progressive dose inhibition against the test organism with total inhibition at 1500 mg/L. Total inhibition of binary mixtures of drysate + aminoforce occurred at 1000 mg/L. At 50%:50% mixture ratios of aminoforce + weedcut combinations, total inhibition occurred at 10,000 mg/L while other ratios exhibited total inhibition at 1,500 mg/L of dehydrogenase activity.

3.4 Inhibitory Concentrations of Individual Chemicals Tested against the Isolates

The experimentally derived toxicity threshold of the respective individual chemicals tested against *L. fusiformis* is shown in Table 3. Results showed that aminoforce was the least toxic chemical to dehydrogenase activity of the isolate with IC_{50} value of 4550.00 ± 62.45 mg/L and drysate the most toxic with IC_{50}value of 1067.33 ± 36.68 mg/L. As individual compounds, the increasing order of toxicity was drysate > weedcut > aminoforce and the Duncan test indicated that the individual IC_{50} of the toxicants differed significantly from one another ($P < 0.05$).

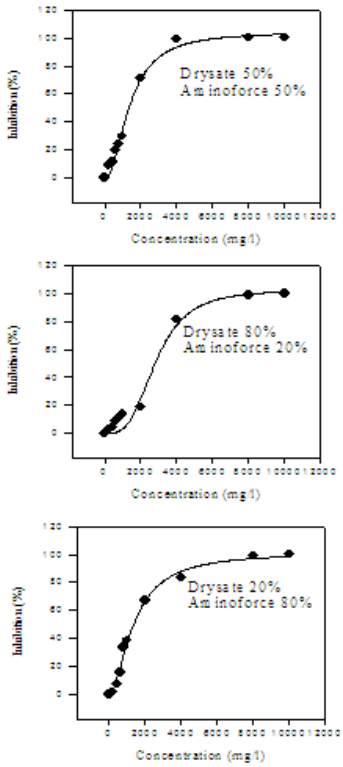

Fig. 2. Inhibitory response of *L. fusiformis* dehydrogenase activity by binary mixtures of aminoforce and drysate

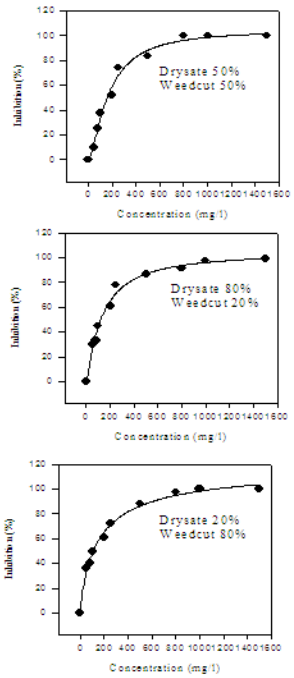

Fig. 3. Inhibitory response of *L. fusiformis* dehydrogenase activity by binary mixtures of drysate and weedcut

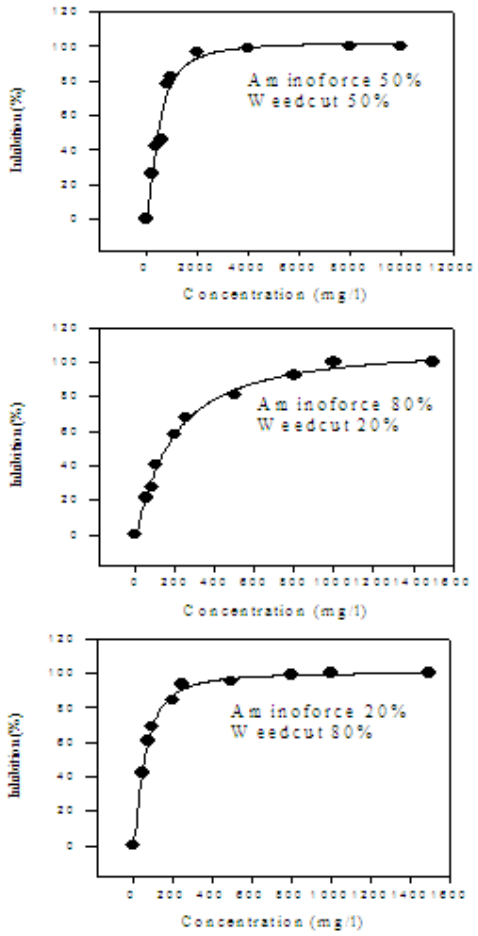

Fig. 4. Inhibitory response of *L. fusiformis* dehydrogenase activity by binary mixtures of aminoforce and weedcut

Table 3. Inhibitory concentrations (IC_{50}) of the individual chemicals to dehydrogenase activities of *L. fusiformis*

Toxicant	Toxicity threshold (IC_{50}) of *L. fusiformis* (mg/L)
Drysate	1067.33 ± 36.68[b]
Aminoforce	4550.00 ± 62.45[d]
Weedcut	2180.00 ± 147.31[c]

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

3.5 Inhibitory Concentrations of the Binary Mixtures

The 24-h toxicity threshold (IC_{50}) of the chemical mixtures and their statistical associations are shown in Tables 4. As binary mixtures of drysate + aminoforce, 24–h IC_{50} obtained showed that 50%:50% combination had the highest toxicity with IC_{50}value of 1,420 ± 72.11 mg/L while 20%:80% of the same mixture with IC_{50} of 1,1350 ± 88.88 mg/L was the least toxic among other ratios evaluated. Thus, their toxicity thresholds are significantly different ($p < 0.05$) using Duncan statistical test method. Drysate + weedcut binary mixtures showed highest toxicity at combined ratios of 80%:20% with IC_{50}value of 141.37 ± 9.42 mg/L and Duncan statistical test indicated statistical difference between the IC_{50} of 80%:20% mixture and the other combination mixture ratios ($p < 0.05$). Other combination ratios of drysate + weedcut evaluated showed similar toxic effect whose IC_{50} values were not

significantly different ($p > 0.05$). Aminoforce + weedcut mixture asserted highest toxicity for 20%:80% and lowest for 50%:50% with IC_{50}values of 65.37 ± 1.18 mg/L and 480.92 ± 15.44 mg/L, respectively. However, IC_{50} values for combination ratios 20%:80% and 80%:20% were not significantly different from each other ($p > 0.05$).

3.6 Isobolographic Representation of Toxicity f Binary Combinations of the Chemicals

Fig. 5 showed isobolographic representations of the various toxic interactions of the binary mixtures of the chemicals tested against *L. fusiformis*. From the results, toxic interactions of drysate + weedcut, and wminoforce + weedcut were synergistic. Drysate + aminoforce combinations showed both synergistic and antagonistic effects.

3.7 Toxic Index (TI) Profile

According to the TI model, synergistic and antagonistic effects were observed in the mixtures as shown in Table (5). The TI values for drysate and aminoforce mixtures was 33.33% antagonistic but 66.66% synergistic. All other binary combinations evaluated were 100% synergistic.

Table4. Inhibitory concentrations (IC_{50}) of the binary combinations of the chemicals to dehydrogenase activities of *L. fusiformis*

Toxicants mixture	Toxicity threshold (IC_{50}) mg/L *l. fusiformis*
Drysate + Aminoforce	
50%: 50%	1420 ± 72.11[a]
80%: 20%	2865 ± 21.57[b]
20%: 80%	11350 ± 88.88[c]
Drysate + weedcut	
50%: 50%	169.27 ± 10.46[b]
80%: 20%	141.37 ± 9.42[a]
20%: 80%	153.41 ± 10.66[a, b]
Aminoforce + Weedcut	
50%: 50%	480.92 ± 15.44[a]
80%: 20%	178.01 ± 2.65[b]
20%: 80%	65.37 ± 1.18[c]

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

Table 5. Toxic index of the test chemicals in binary mixtures and their respective interactions on dehydrogenase activity of the bacteria *L. fusiformis* according to TI Model

Toxicant Mixtures	*L. fusiformis*	
	TI	Effect
Drysate + Aminoforce		
50%:50%	0.840	Synergistic
80%:20%	2.346	Antagonistic
20%:80%	0.512	Synergistic
Drysate + Weedcut		
50%:50%	0.124	Synergistic
80%:20%	0.115	Synergistic
20%:80%	0.092	Synergistic
Aminoforce + Weedcut		
50%:50%	0.171	Synergistic
80%:20%	0.049	Synergistic
20%:80%	0.026	Synergistic

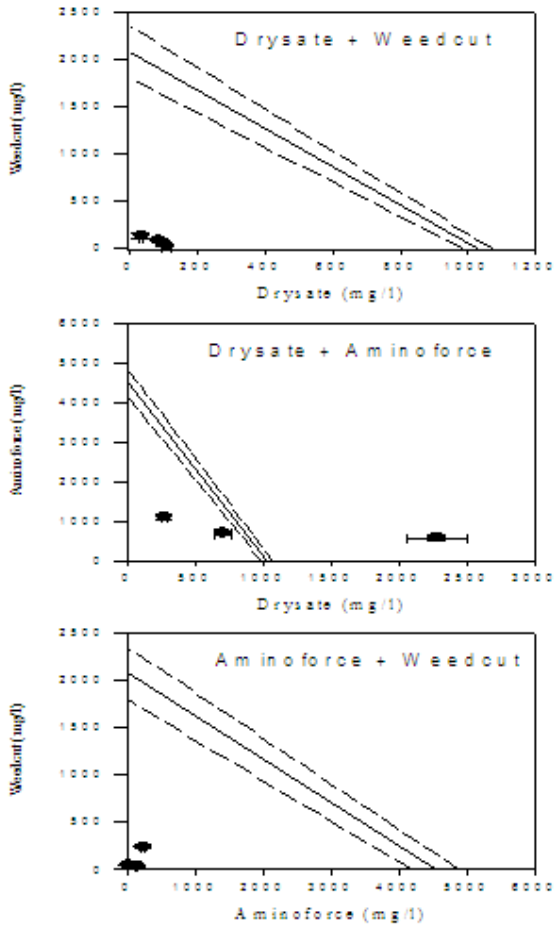

Fig. 5. Isobologram representation of inhibitory effects of binary mixtures of drysate + weedcut, drysate + aminoforce and aminoforce + weedcut to dehydrogenase activity of *L. fusiformis*. The thick line represents line of additivity while the dotted lines are 95% confidence interval

4. DISCUSSION

The use of herbicides for clearing weeds in preparation for farming is now a common practice. Farmers major interest centers on getting rid of weeds completely. They are ignorant that some beneficial indigenous microorganisms are harmed by that exercise. Drysate, weedcut and aminoforce herbicides in the environment adversely affects the activities of the *L. fusiformis*.

Nkamigbo et al.[17] reported that among the enzymes in the soil environment, dehydrogenases (DHA) are one of the most important and used as an indicator of overall soil microbial activity. Toxicity of glyphosate to DHA of bacteria and other microorganisms have been reported by Nweke et al.[14] and Nkamigbo et al.[17]. The responses of *Lysinibacillus fusiformis* to the stresses of drysate, weedcut and aminoforce as single pollutants are shown in Fig. 1. Weedcut had a biphasic effect on the DHA of *L. fusiformis.* Hormesis occurred at concentration up to 1,500 mg/L of weedcut; total inhibition at 10,000 mg/L. The observed hormetic response could be due to increase in respiration at low concentrations of the chemical by the isolate portraying the organism as a competent one in making use of the pollutant as a substrate for carbon source. Total inhibition at such high concentration of 10,000 mg/L of the herbicide implies that the compound is actually not toxic when compared to total inhibition by drysate at 3,000 mg/L. This finding disagreed with the report of Adomako and Akyeampong [10] that

paraquat treatment resulted in reduction in the bacterial population in the soil at a concentration of 250 µg/L, which is far lower than the concentration of 1,500 mg/L hormetic response limit of weedcut studied. All other herbicides progressively repressed the growth of the test organism. Inhibition by aminoforce also reached saturation at 10,000 mg/L. Drysate is highly toxic have reached saturation at low dose of 3000 mg/L concentration. This finding does not also agree with the report of Nweke et al.[14] that hormetic effects of glyphosate at low doses on the DHA was experienced by pure culture of *Rhizobium* species. However, the microbial community was more tolerant to the formulated aminoforce than the drysate. The order of decreasing toxicity is drysate > weedcut > aminoforce. Duncan test indicated significant statistical difference in their IC_{50}($P < 0.05$).

Herbicides may persist in an environment depending on the application rate and dose. They cannot exist in isolation. There must be an interaction with one another to either enhance or diminish hazardousness. Most information on toxicity are those effects received from single compounds. Knowledge of possibilities of interactions of contaminants will lead to better preparedness on the issues of environmental management and decision making. The importance of studying toxicity of mixtures of compounds cannot be overemphasized. Toxicity of the fixed ratio binary mixtures showed progressive inhibition of dehydrogenase activity with drysate + aminoforce combination attaining total inhibition at 10,000 mg/L on *L. fusiformis*. The ability of the test organism to tolerate the toxicant mixture up to 10,000 mg/L maybe the reason glyphosate is mixed with 2,4-D for better yield [13] because of the low toxicity when in mixture. The implication is that the rhizobacterial that could be beneficial to plant will not be affected adversely, rather the organism could use the chemical as substrate for carbon source. Good to note that the active ingredient in aminoforce – 2,4-D rapidly degrades to derivatives that are more toxic to the parent material [14]. Total inhibition to DHA resulted from the mixture of drysate + weedcut at 1,500 mg/L.The toxicity could have been enhanced by the presence of drysate in the mixture. This is not in line with the findings of Nweke et al.[14] that at low concentration of chemicals, growth is stimulated.

Drysate with IC_{50}value of 1,067 ± 36.68 mg/L was the most toxic and the least was aminoforce with IC_{50}value of 4,550.00 mg/L. The lesser the IC_{50}, the higher the toxicity of the compound. This corresponds to the toxicity trend of the chemicals of this study where the most toxic was drysate and the least was aminoforce. The inhibitory concentration of the binary mixtures of drysate + aminoforce showed that 50% /50% combination induced highest toxicity to *L. fusiformis* with IC_{50}value of 1,420.00 ± 72.11 mg/L because one of the components is highly toxic, thus improved the toxicity of the mixture. That of 20%/ 80% mixture ratio with IC_{50} of 1,1350.00 ± 88.88 mg/L was the least toxic to organism because aminoforce is more in the mixture and so reduced the mixture toxicity. Drysate + weedcut binary mixtures showed highest toxicity to *L. fusiformis* at combined ratios of 80%:20% with IC_{50} values 141.37 ± 9.42 mg/L and Duncan test indicated that the effect is significantly different from other combination ratios of the mixture ($p < 0.05$). Aminoforce + weedcut showed highest toxicity for 20%/80% mixture with IC_{50} value of 62.37 ± 1.18 mg/L for the isolate. This may be because of the presence of weedcut in the combination being a higher toxic compound than aminoforce.This agreed with the findings of Roberts et al.[18] that reported inhibition of some soil microorganisms at as low as 5 mg /L (0.05%) of paraquat meaning that paraquat is highly toxic. From the result, the combination with higher percentage of paraquat presented higher toxicity. Paraquat is more toxic than glyphosate and also than aminoforce [14] but glyphosate was found to be more toxic than paraquat while aminoforce has been the least toxic in this study. The IC_{50}value for combination ratio of 80% /20% for drysate + weedcut and 50% /50% for drysate + aminoforce and aminoforce + weedcut are not significantly different ($p > 0.05$). This indicates that their toxicity threshold is the same. The inhibition of dehydrogenase activity at low doses of drysate observed in this study agreed with the findings of Busse [5] who reported that glyphosate exhibits higher toxicity in soil-free media. From the findings in this study, drysate is highly toxic to the rhizobacterium and so disagreed with the publications of Gimsing et al.[19] and Muoneke et al.[20] who reported that several bacterial species have been demonstrated to grow on glyphosate and its biodegraded intermediates.

The interactive effects of binary mixtures of the herbicides were analyzed using isobologram as a tool. Combination of drysate + weedcut and aminoforce + weedcut gave synergistic effect on DHA of the isolates. This could be that drysate

enhanced toxicity of weedcut in the mixture and weedcut enhanced that of aminoforce and thus resulted to synergism. Drysate + aminoforce combination showed antagonistic effect on the DHA implying that in mixtures. They do not have any adverse effect on the activities of the microorganism rather the harmful effect of drysate is being reduced by the presence of the aminoforce. This may be the reason farmers apply the mixtures of the two herbicides (drysate and aminoforce). Most of the toxicity reports against bacteria dealt with herbicides as a single agent. However, in the natural environment, microorganisms are exposed to mixtures of chemicals which have toxicities different from those of their individual components. These chemicals may also interact to modulate the toxicity of each other in a mixture. This has been established in this study with the formulated herbicides tested. Glyphosate modulated the toxicity of weedcut, aminoforce and vice versa producing synergistic or antagonistic effects. This modulation however, seem to be dependent on the relative proportions of the most toxic and least toxic components [14]. The fact that the toxic index of the test chemicals in binary mixtures and their respective interactions on dehydrogenase activity of the test organism produced 88.89% synergism and 11.11% antagonism in this study revealed that the joint action of the combination mixtures against *L. fusiformis* dehydrogenase activity was synergistic. This implied that herbicides are very dangerous to the test organism especially when they are in mixtures or repeatedly applied and therefore have the opportunity to interact.

5. CONCLUSION

This study evaluated the joint effects of binary mixtures of selected herbicides (drysate, weedcut and aminoforce)on *Lysinibacillusfusiformis* isolated from *Oryzasativa* plant. Inhibition of dehydrogenase activity in this test organism increased with increasing concentrations and the percentage of the toxicants in the mixtures. Although some researchers have reported glyphosate (active ingredient in Drysate) to be safe. It was rather found to be highly toxic compared to other studied herbicides. The results of this study also suggested that in combination with some other herbicides, the mixtures could interact synergistically inducing more harm to the soil microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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