



# Invitro Toxicity of Binary Mixtures of Glyphosate and 2, 2 Dichlorovinyl Dimethyl Phosphate on Bacterial Isolates

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The *in vitro* toxicity of glyphosate (Gly) and 2, 2 Dichlorovinyl dimethyl phosphate (DDVP) single compound and binary mixtures was assessed against *Pseudomonas sp.* and *Bacillus sp.* isolated from Otamiri River, Imo state, Nigeria was investigated. The toxicity response was assessed using the inhibitory effect of the single and binary mixtures on isolates dehydrogenase activity; and 2,3,5 triphenyltetrazolium chloride (TTC) was used as the artificial electron acceptor. The binary mixtures were composed using fixed ratios of glyphosate and 2, 2 Dichlorovinyl dimethyl phosphate in ratios of 20% Gly:80% DDVP, 40% Gly: 60% DDVP, 50% Gly: 50% DDVP, 60% Gly: 40% DDVP and 80% Gly: 20% DDVP. Results obtained showed that the isolates exhibited different degrees of logistic and sigmoidal toxicity trends with areas of hormesis at low concentrations of the toxicants. Furthermore, isobolographic analysis on the toxic interaction of the mixtures presented both synergism and antagonism, based on the relative ratio of the component mixtures. Increasing concentration of glyphosate in the binary mixture caused a shift in the interaction effect from antagonism to synergism. Our findings showed that isolates exhibited tolerance to glyphosate and 2,2 dichlorovinyl dimethyl phosphate and their binary mixtures exposure at concentration range of 0-

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1000mg/L; above which has deleterious effects on the aquatic organisms. It is evident that there are considerable differences in pesticide sensitivity among the bacterial species and that the presence of glyphosate and 2, 2-dichlorovinyl dimethyl phosphate in the aquatic environment may present toxicological risk to microbial diversity.

**Keywords:** Glyphosate; DDVP; toxicity; Hormesis; Binary mixtures.

## 1. INTRODUCTION

The rapid increase in global population in recent years has created a lot of pressure on the existing agricultural systems. According to Saravi and Shokrzadeh [1], world populations grow by an estimated rate of about 97 million each year. By 2050, the world population is estimated to exceed 10 billion; this population explosion is paralleled by increased demand for food to feed the teeming population [2]; [3]. This has resulted to overdependence on modern farming methods and inputs such as insecticides, fumigants, herbicides, fertilizers to boost production. An estimated 2 million tons of pesticides are used globally as 47.5% for herbicides, 29.5% for insecticides, 17.5% for fungicides, and 5.5% for other pesticides [4-5]. The effects of pesticides on the growth of various groups of soil microbial community are not easily predicted. Some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on nitrifying bacteria [6]. Glyphosate [N-(phosphonomethyl)glycine] is a widely applied non-specific organophosphate pesticide which act by inhibiting the photosynthesis process of terrestrial and aquatic plants [7]. It is a widely used herbicide in agriculture against perennial, annual weeds and in silviculture, domestic gardens, and urban areas [8]. Glyphosate has been sold under trade names such as Roundup, Force Up, Rodeo, Glypro, GGlyphomax, Touchdown, etc [9].

Glyphosate acts by targeting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and obstructs the aromatic amino acid biosynthesis in the shikimate pathway [10]. The inhibition of EPSPS retards the synthesis of essential secondary metabolites and proteins; and distorts vital energy pathways in soil microbes and plants [11]. Recent research have focused on the toxicity of glyphosate and interaction with other toxicants. [12] reported that glyphosate alters the soil texture and microbial diversity by reducing the microbial richness and increasing the population of phytopathogenic fungi. In another study, [13] reported additive

interactions of formulated glyphosate with the phenolic compounds.

Also, studies have proven that a greater amount of these pesticides do not end up in their intended locations and are dispersed into the ecosystem to non-target organisms through various mechanisms such as run off, erosion deposition by wind, biomagnification, and bioaccumulation [14].

2,2-dichlorovinyl dimethyl phosphate (DDVP) also known as Dichlorvos is an organophosphate insecticide cum pesticide [15]. Its trade names include DDVP, Dedevap, Sniper and Nuvan etc. [16]; [17]. Dichlorvos is used as a household insecticide and applied in agriculture as an organophosphate pesticide [18]. Dichlorvos toxicity is mediated by an irreversible inhibition of neural acetylcholinesterase; which promotes accumulation of acetylcholine in synapses with resultant disruption of nerve function [19]. The altered cholinergic neurotransmission in the parasympathetic autonomic nervous system results to perspiration, nausea, lacrimation, vomiting, diarrhea, excessive bronchial secretion or even death [20]. DDVP application has been reported to decrease soil microbial counts and toxicity to microorganisms which may be beneficial to cultivated plants [21]. However, microbes such as *Cunninghamella*, *Fusarium*, *Talaromyces*, *Aspergillus*, *Penicillium*, *Ochrobium*, *Pseudomonas*, *Bacillus*, and *Trichoderma* have been isolated and reported to possess DDVP-degrading [22]. The genus *Pseudomonas* is well known for its metabolic versatility and genetic plasticity; capable of utilizing a wide range of inorganic compounds and of living under diverse environmental conditions. Consequently, they are ubiquitous in soil and water ecosystems and are important as plant, animal, and human pathogens [23]. Some species of *pseudomonas* such as *P. aeruginosa* plays an important role in the environment by utilizing pesticides as its carbon source and energy thereby viable for the biodegradation and bioremediations of toxic compounds [24]. The global use of these pesticides implies the environment is constantly abashed by a

multiplicity of pesticide mixtures and also other contaminants like heavy metals and phenolic derivatives [25]. Consequently, non-target organisms are constantly exposed to single or to mixture of various pesticides, often in low concentrations, that may evoke similar effects irrespective of their various origins. Hence, prolonged exposure to low doses of pesticide mixtures is a more realistic scenario that may give rise to multiple potential interactions amongst different pesticides. The novelty of this work is sacrosanct on its assessment of the *in vitro* toxicity of binary mixtures of glyphosate and 2,2 Dichlorovinyl dimethyl phosphate on *Pseudomonas sp.* and *Bacillus sp.* isolated from Otamiri River and their possible interactive effect.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study area is one of the main Rivers in Imo state. The river runs south from Egbu past Owerri and through Nekede, Ihiagwa, Eziobodo, Olokwu, Umuisi, Mgbirichi and Umuagwo to Ozuzu in Etche, in the Rivers State, from where it flows to the Atlantic Ocean. The Otamiri watershed spans up to 10,000 square kilometers with an average annual rainfall of 2,375 millimeters (138 in). This watershed is mostly covered by depleted rain forest vegetation, with mean temperatures of 27 °C (81 °F) throughout the year.

The Otamiri meets the Nworie River at Nekede in Owerri, a river about 9.2 kilometers (5.7 mi) long. The Nworie River is subject to intensive human and industrial activities and is used as a source of drinking water by the inhabitants of the area when the public water system fails. The Nworie is polluted by chemical, organic wastes, and effluent from nearby farms. Waste management in Owerri is inefficient and contributes to pollution of the river.

### 2.2 Materials

The reagents and media used in this study were 2, 3, 5-triphenyltetrazolium chloride (TTC) (Sigma-Aldrich, USA), n-Butanol (BDH, England), Distilled water, Nutrient agar (Titan Biotech, India), Nutrient broth (Titan Biotech, India), Mac Conkey agar (Titan Biotech, India). The pesticide used was Formulated glyphosate (Force up), while insecticide was Dichlorvos (DD

Force) (JAT, Lebanon). All other reagents used were of analytical grade.

### 2.3 Sample Collection

Water samples collected in sterilized plastic bottles of 500 ml capacity were used for bacterial analysis. The samples, after collection were properly labeled and stored in cooler containing icepacks to maintain stable temperature of 4°C and immediately transported to the laboratory for further analysis.

### 2.4 Isolation and Purification of Isolated Bacteria

Water samples collected in sterilized plastic bottles were serially diluted to 10<sup>-3</sup> and 10<sup>-4</sup> dilutions, and 0.1ml of aliquot was inoculated unto sterile petri-dish containing 20ml of nutrient agar and MacConkey agar by pour plate method for 24hrs. The isolates obtained were further purified on nutrient agar plates and characterized using standard microbiological and biochemical methods. Identifications of organisms followed the schemes described by [26].

#### 2.4.1 Preparation of test organisms for toxicity test

The isolates were purified and grown to mid exponential phase in nutrient broth (TM Media) on a rotatory incubator (Marrienfeld, Germany) at room temperature (28 ± 2°C) and 150 rpm. The cells were later harvested by centrifugation at 4000 rpm for 10mins and washed thrice in sterile distilled water. The washed cell was re-suspended in sterile distilled water and standardized using spectrophotometer (UV-Visible Spectrophotometer, 752N) to obtain an optical density of 0.1 at 540nm. An aliquot of 0.1 ml of the cell suspension was used as inoculum in the dehydrogenase assay.

### 2.5 Binary Mixture Ratios

The binary mixtures contained glyphosate (as active ingredient in formulated glyphosate) and 2,2 Dichlorovinyl dimethyl phosphate (as active ingredient in Dichlorvos insecticide). The binary mixtures were composed *In vitro* using fixed ratio of 20% Gly + 80% DDVP, 40% Gly + 60% DDVP, 50% Gly + 50% DDVP, 60% Gly + 40% DDVP and 80% Gly + 20% DDVP with a stock concentration of 5000mg/L of each toxicant. The

binary mixtures were further graded into varying doses for toxicity studies.

## 2.6 Toxicity Assay Using Inhibition of Dehydrogenase Activity

The assay was carried out using inhibition of dehydrogenase activity as measure of toxicity as reported by [27]. The toxicity of glyphosate and 2, 2-dichlorovinyl dimethyl phosphate on *Pseudomonas sp.* was determined in 2ml volume containing X4-strength nutrient broth, 2, 3, 5-triphenyltetrazolium chloride and stock concentration of glyphosate and/or Dichlorovinyl dimethyl phosphate mixtures and requisite volumes of distilled water. Total dehydrogenase activity was assayed using TTC (BDH England) as the artificial electron acceptor, which was reduced to the red-colored triphenyl-formazan (TPF). The set-up was in triplicate glass tubes consisting of 0.5ml nutrient broth medium amended with toxicant mixtures and requisite volumes of distilled water, pre-incubated on a rotary incubator (150 rpm) at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 30 mins; and 0.1 ml of the standardized inoculum suspensions were inoculated into tubes. Thereafter, 0.1 ml of 0.1 % (w/v) TTC in deionized distilled water was added to each tube to obtain final concentrations of 0-3000 mg/L. The controls consisted of the isolates and the media void of toxicants; the tubes were incubated in the dark for 24 hours at  $28 \pm 2^\circ\text{C}$ . Furthermore, formazan produced, was extracted by shaking in 4ml of n-Butanol. The absorbance of extracted formazan was determined at 500nm using the spectrophotometer. The percentage inhibition of dehydrogenase activity of the isolates was calculated relative to control.

## 2.7 Data Analysis

The percentage inhibition of dehydrogenase activity of the isolates was 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), and Weibullcum(a, b, c, d) (eqn 4), The dose-response data were fitted to obtain their respective  $EC_{50}$  which is the concentrations of the toxicants that inhibited 50% of dehydrogenase activity of the isolate. All Curve fittings were done using Sigma plot 10 and Table Curve 2D v5.01.

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

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  $EC_{50}$ , c is parameter determining the relative slope at  $EC_{50}$ .

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d} \quad 3$$

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

$$y = a \left[ 1 - \exp \left[ - \left[ \frac{x + c(\ln 2)^{1/d} - b}{c} \right]^d \right] \right] \quad 4$$

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

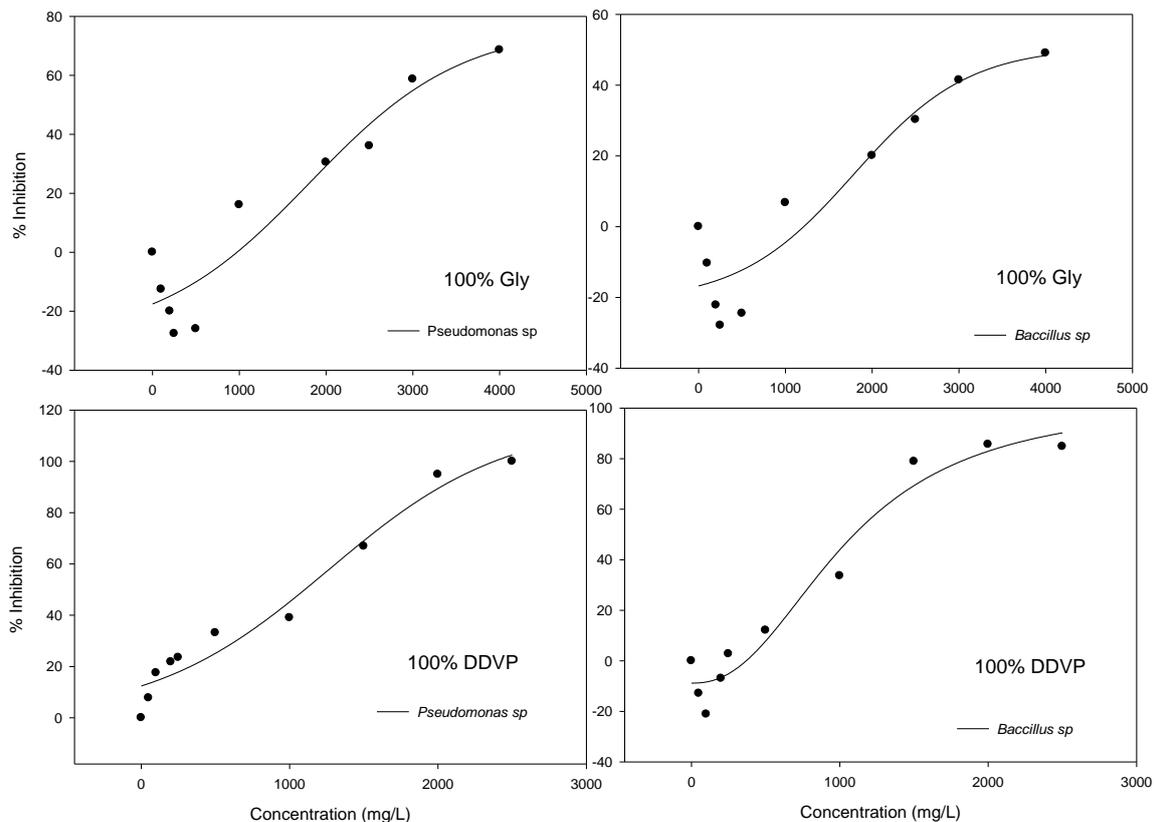
## 3. RESULTS AND DISCUSSION

**Toxicity of formulated glyphosate and 2,2-dichlorovinyl dimethyl phosphate (DDVP) to *Pseudomonas sp.* and *Bacillus sp.*, isolates from Otamiri river:** Results presented in Fig. 1 shows the inhibitory effect of glyphosate and DDVP to the dehydrogenase activity of *Pseudomonas sp.* and *Bacillus sp.*, isolates obtained from Otamiri River. The toxicity of glyphosate to the two isolates presented below exhibited a biphasic toxicity trend: with hormetic response of the isolates to glyphosate at low doses of 0-1000mg/L. However, the isolates dehydrogenase activities were progressively inhibited beyond the threshold concentration of 1000 mg/L. Above this concentration, toxicity of glyphosate was concentration dependent and closely fitted into a mathematical sigmoidal relationship. Furthermore, the toxicity of DDVP was observed to be largely biphasic and sigmoidal with a narrow hormetic effect was seen in the response of *Bacillus sp.* while to *Pseudomonas sp.*, it was mostly inhibitory even at low concentrations. The  $EC_{50}$  of glyphosate were  $3006.57 \pm 180.66\text{mg/L}$  and  $3960.03 \pm 198.00\text{mg/L}$  against *Pseudomonas sp.* and

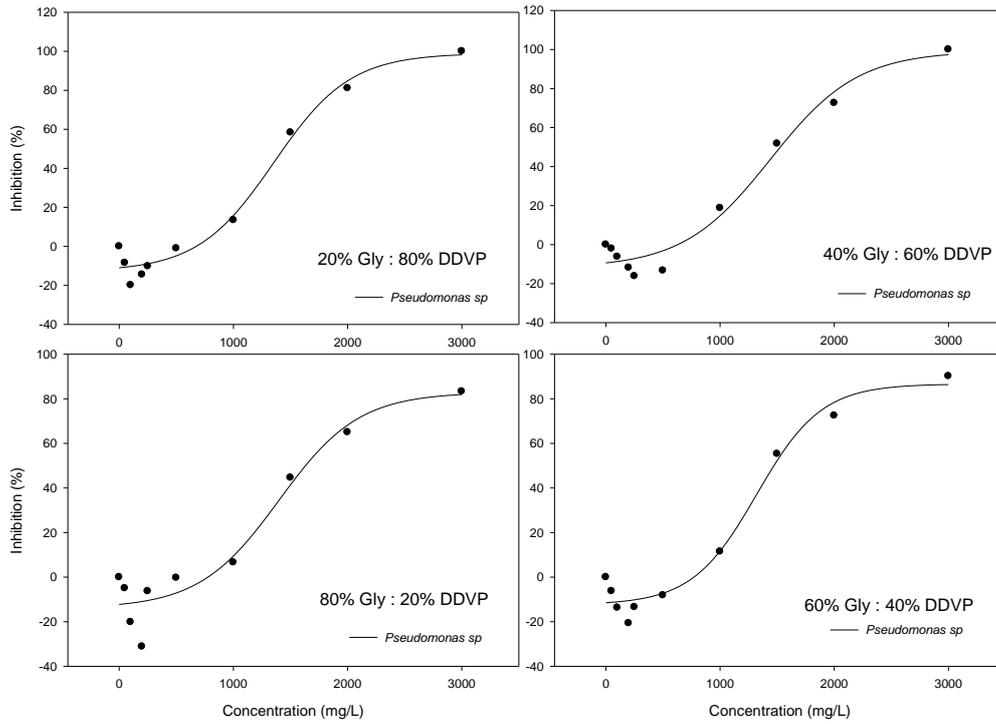
*Bacillus sp.* respectively. While for DDVP, it was  $974.52 \pm 97.45$ mg/L and  $1121.39 \pm 100.93$ mg/L respectively.

**Toxicity of glyphosate and Dichlorvos (2,2-dichlorovinyl dimethyl phosphate (DDVP) binary mixtures to *Pseudomonas sp.* isolate from Otamiri river:** The results presented in Fig. 2 shows the toxicity of glyphosate and DDVP binary mixtures using arbitrary concentration ratios of 20% Gly + 80% DDVP, 40% Gly + 60% DDVP, 60% Gly + 40% DDVP and 80% Gly + 20% DDVP to *Pseudomonas sp.* dehydrogenase activity. Result obtained showed that the toxicity response exhibited a sigmoidal relationship, Toxicity response at low dose of the mixtures was largely hormetic, and the hormetic range was between 0-800 mg/L. The EC<sub>50</sub> value obtained for the binary mixtures was  $1416.22 \pm 90.62$ ,  $1416.22 \pm 55.78$ ,  $1462.64 \pm 74.26$  and  $1632.38 \pm 97.94$  mg/L for the mixtures 20% Gly + 80% DDVP, 40% Gly + 60% DDVP, 60% Gly + 40% DDVP and 80% Gly + 20% DDVP respectively.

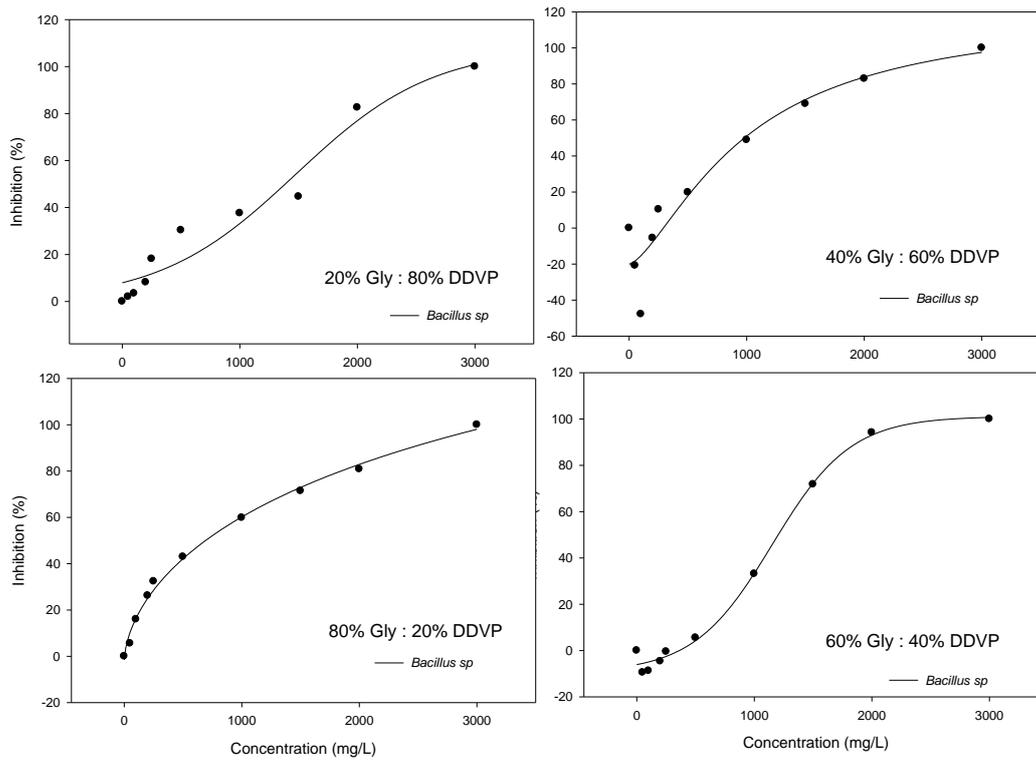
**Toxicity of glyphosate and Dichlorvos (2,2-dichlorovinyl dimethyl phosphate (DDVP) binary mixtures to *Bacillus sp.* isolate from Otamiri river:** The results presented in Fig. 3, shows the toxicity of glyphosate and DDVP binary mixtures using arbitrary concentration ratios of 20% Gly + 80% DDVP, 40% Gly + 60% DDVP, 60% Gly + 40% DDVP and 80% Gly + 20% DDVP to *Bacillus sp.* dehydrogenase activity. Response plots of *Bacillus sp.* to the different binary mixture ratios of glyphosate and DDVP showed that the toxicity was logistic in the mixtures (40% Gly + 60% DDVP, and 80% Gly + 20% DDVP) and sigmoidal in the mixtures 20% Gly + 80% DDVP and 60% Gly + 40% DDVP. Hormetic response was observed in the 40% Gly + 60% DDVP and 60% Gly + 40% DDVP, mixture. The EC<sub>50</sub> value obtained for the binary mixtures was  $1276.85 \pm 47.68$ ,  $978.75 \pm 78.30$ ,  $1203.49 \pm 132.38$  and  $692.11 \pm 41.53$  mg/L for the mixtures 20% Gly + 80% DDVP, 40% Gly + 60% DDVP, 60% Gly + 40% DDVP and 80% Gly + 20% DDVP respectively.



**Fig. 1. Toxicity of formulated glyphosate and 2,2-dichlorovinyl dimethyl phosphate (DDVP) to *Pseudomonas sp.* and *Bacillus sp.* isolates from Otamiri river**



**Fig. 2. Toxicity of binary mixtures of formulated glyphosate and 2,2-dichlorovinyl dimethyl phosphate (DDVP) to *Pseudomonas sp.* isolate from Otamiri river**



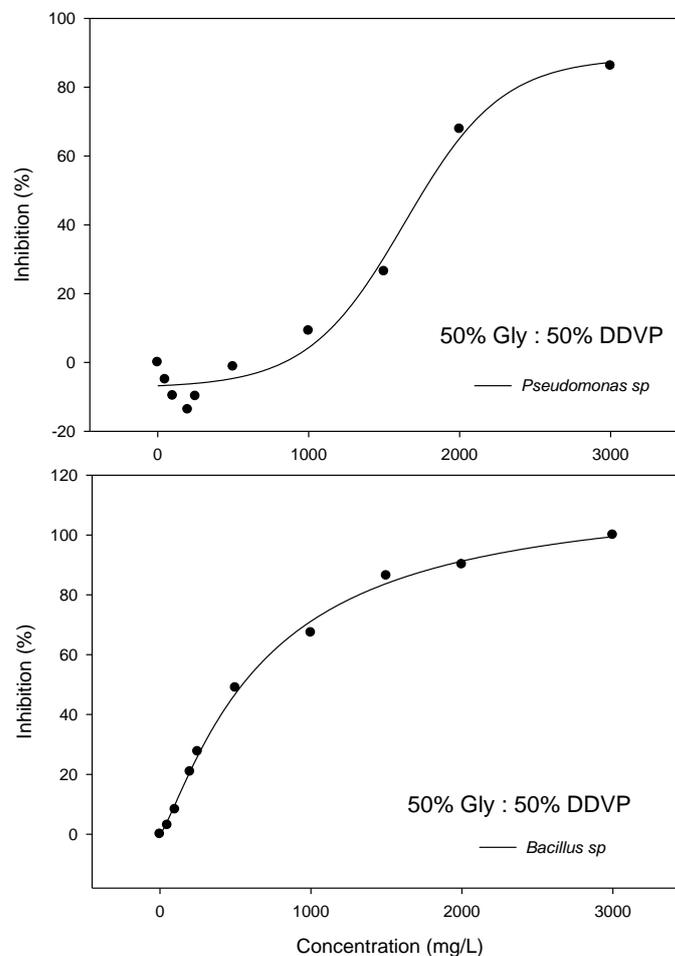
**Fig. 3. Toxicity of binary mixtures of formulated glyphosate and 2,2-dichlorovinyl dimethyl phosphate (DDVP) to *Bacillus sp.* isolate from Otamiri river**

**Toxicity of Equi-ratio binary mixtures of formulated glyphosate and 2,2-dichlorovinyl dimethyl phosphate (DDVP) to *Pseudomonas sp.* and *Bacillus sp.* Isolates:** The results presented in Fig. 4, shows the toxicity of glyphosate and DDVP equi-ratio binary mixtures using arbitrary concentration ratios of 50% Gly + 50% DDVP to *Pseudomonas sp.* and *Bacillus sp.* dehydrogenase activity. The equi-ratio binary mixture was hormetic against *Pseudomonas sp.*, while the 50% Gly + 50% DDVP binary mixture was more toxic against *Bacillus sp.* with EC<sub>50</sub> value of 544.32 ± 38.10 mg/L.

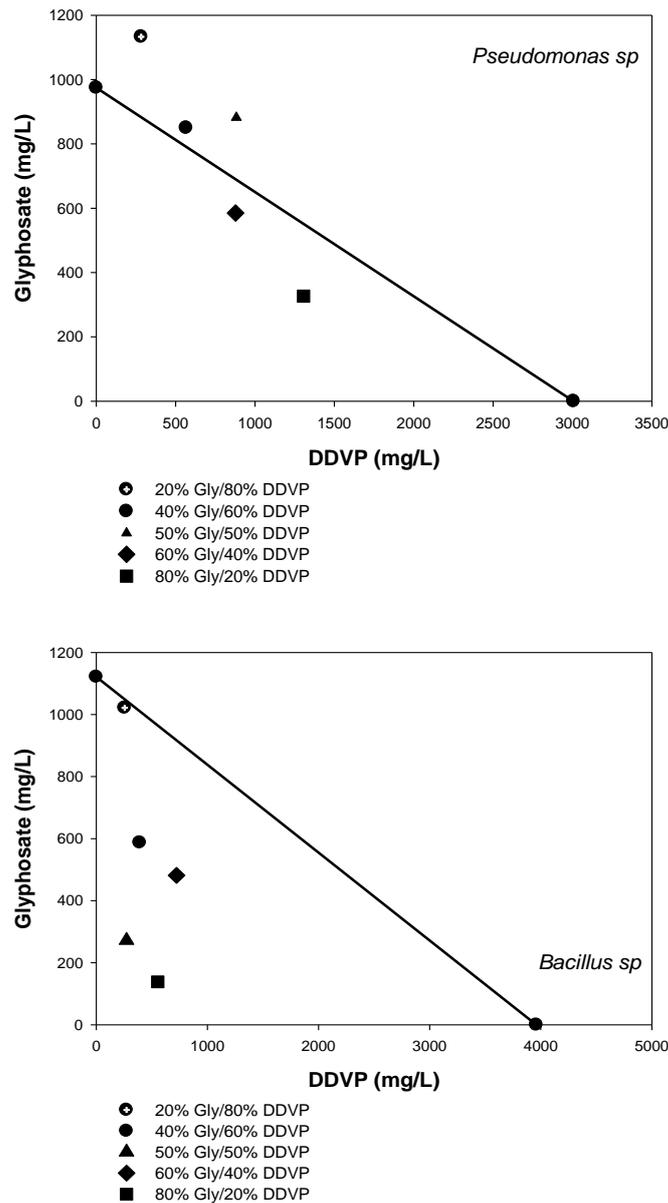
**EC<sub>50</sub> isoboles of glyphosate and DDVP as binary mixtures against dehydrogenase activity of *Pseudomonas sp.* and *Bacillus sp.* obtained from Otamiri river:** In the present study, isobolographic analysis of the joint effect of glyphosate + DDVP binary mixtures against

*Pseudomonas sp.* indicated synergistic, antagonistic, and additive effects for different ratios of the mixtures against the test isolates. The isobolograms (Fig. 5) showed that the toxicity of glyphosate/DDVP mixtures using our study ratios of 60% Gly/40% DDVP and 80% Gly/20% DDVP were synergistic with the values lying below the additivity line, while the 20% Gly/80% DDVP, 50% Gly/50% DDVP mixtures were antagonistic. However, the 40% Gly/60% DDVP was marginally additive, lying slightly above the additivity line.

The isobologram for the toxicity of the binary mixtures of Glyphosate + DDVP against *Bacillus sp.* (Fig. 5) indicated mostly synergistic interaction for the mixture ratios of 40% Gly/60% DDVP, 50% Gly/50% DDVP, 60% Gly/40% DDVP and 80% Gly/20% DDVP; while the 20% Gly/80% DDVP mixture was marginally additive.



**Fig. 4. Toxicity of Equi-ratio binary mixtures of formulated glyphosate and 2,2-dichlorovinyl dimethyl phosphate (DDVP) to *Pseudomonas sp.* and *Bacillus sp.* isolates from Otamiri river**



**Fig. 5. The EC<sub>50</sub> isoboles of glyphosate and DDVP as binary mixtures against dehydrogenase activity of *Pseudomonas sp.* and *Bacillus sp***

**Threshold eco-toxic concentration (EC<sub>50</sub>) of Single and binary mixtures of glyphosate and DDVP on bacterial isolates:** Table 1 shows the threshold toxic concentration of the single compound and their binary mixtures to the dehydrogenase activity of the isolates *Pseudomonas sp.* and *Bacillus sp.* obtained from Otamiri river. The toxicants exhibited varying degree of toxicity to the isolates as shown in the EC<sub>50</sub> values obtained. Comparison of the toxicants using EC<sub>50</sub> ranking, DDVP single

compound was more toxic than glyphosate and the 50% Gly + 50% DDVP and 80% Gly + 20% DDVP mixture ratios were the most toxic binary mixtures to *Bacillus sp.* with EC<sub>50</sub> values of 544.32 ± 38.10 mg/L and 692.11 ± 41.53 mg/L respectively. However, against *Pseudomonas sp.*, 20% Gly + 80% DDVP and 40% Gly + 60% DDVP mixtures were the most toxic with EC<sub>50</sub> values of 1416.22 ± 90.62 mg/L and 1416.22 ± 55.78 mg/L respectively.

**Table 1. Threshold eco-toxic concentration (EC<sub>50</sub>) of Single and binary mixtures of glyphosate and DDVP on bacterial isolates**

| Toxicants          | Threshold eco-toxic concentration (EC <sub>50</sub> ) (mg/L) |                     |
|--------------------|--|---------------------|
|                    | <i>Pseudomonas sp.</i>                                       | <i>Bacillus sp.</i> |
| Glyphosate         | 3006.57 ± 180.66   | 3960.03 ± 198.00    |
| DDVP               | 974.52 ± 97.45   | 1121.39 ± 100.93    |
| 20% Gly + 80% DDVP | 1416.22 ± 90.62  | 1276.85 ± 47.68     |
| 40% Gly + 60% DDVP | 1416.22 ± 55.78  | 978.75 ± 78.30      |
| 50% Gly + 50% DDVP | 1764.13 ± 141.13   | 544.32 ± 38.10      |
| 60% Gly + 40% DDVP | 1462.64 ± 74.26  | 1203.49 ± 132.38    |
| 80% Gly + 20% DDVP | 1632.38 ± 97.94  | 692.11 ± 41.53      |

Values presented are mean ± standard deviation of 3 determinations

The toxicity of glyphosate and 2, 2-dichlorovinyl dimethyl phosphate binary mixtures on *Pseudomonas sp.* and *Bacillus sp.* isolates from the Otamiri River was investigated. Glyphosate and 2, 2-dichlorovinyl dimethyl phosphate as single compounds and mixtures demonstrated tolerance and varying degree of toxicity to the isolates obtained from Otamiri River. The response of *Bacillus sp.* and *Pseudomonas sp.* to pesticides exposure were biphasic; with a hormetic response at low doses of 0-1000mg/L. At higher concentrations, the toxic effect of glyphosate on dehydrogenase activities of the isolates was progressive inhibitory beyond the threshold concentration of 1000 mg/L. Above this concentration, the toxicity of glyphosate was concentration-dependent and closely fitted into a mathematical sigmoidal relationship.

The isolate *Pseudomonas sp.* exhibited a biphasic toxicity response to glyphosate and DDVP binary mixtures. At low doses, the responses were largely hormetic to the tested binary mixtures.

The hormetic doses of the binary mixtures of glyphosate and DDVP using arbitrary concentration ratios of 20% Gly + 80% DDVP, 40% Gly + 60% DDVP, 50% Gly + 50% DDVP, 60% Gly + 40% DDVP and 80% Gly + 20% DDVP were largely between 0-1000 mg/L of the mixture. Hormetic range increased with increasing percentage component of glyphosate in the mixture; beyond this range, the mixtures were inhibitory to the dehydrogenase activity of the isolates.

Furthermore, glyphosate and DDVP binary mixtures were strongly inhibitory against *Bacillus sp.* dehydrogenase activity. The toxicity of the mixtures was dose-dependent and significantly increased with increasing concentration of the mixture, with exceptions in 40% Gly: 60% DDVP

and 60% Gly: 40% DDVP mixture which were hormetic at low doses. Hormetic response of microorganisms against pesticides toxicity has been widely reported [28-30], [27], [13]. These responses involve a complex process which is genetically and physiologically controlled [28]. Tolerance to pesticides has been attributed to physiological changes that induce alternative metabolic pathways to bypass a biochemical reaction inhibited by a specific pesticide [31]; Permanent resistance, on the other hand, depends upon genetic modifications, inherited by the subsequent generation of microbes [32-33]. The findings of the present study are consistent with [27]; which reported the hormetic response of *Rhizobium* species to low doses of glyphosate. Also, stimulatory responses of soil microbial community to glyphosate have been reported [34-35]. The observed ability of the isolates to utilize glyphosate and the mixtures as growth substrate may be attributed to the induction of degradative enzymes, which have been reported in several studies in different environmental matrices (*Achromobacter sp.* MPK 7A, *Comamonas odontotermitis* P2, *Ochrobactrum intermedium* Sq20, and *Pseudomonas sp.* 4ASW) in contaminated niches via enrichment approach [36-38]. Also, species such as *Arthrobacter atrocyaneus* ATCC 13752, *Alcaligenes sp.* GL, *Arthrobacter sp.* GLP-1, *Geobacillus caldoxylosilyticus* T20, and *Pseudomonas sp.* PG2982 has been reported to utilize glyphosate as a growth substrate [39-41]. Our study also closely agrees with [42]; which reported a concentration and time-dependent inhibition of soil's microbial communities by organophosphorus insecticides (dimethoate, diazinon, and Malathion). Dichlorvos toxic effect is mediated through irreversibly inhibition of neural acetylcholinesterase [19]. The inhibition promotes the accumulation of acetylcholine in synapses with disruption of nerve function [19]. The chief intermediates of glyphosate

metabolism have been identified as Aminomethylphosphonic acid (AMPA), Acetyl-glyphosate and sarcosine and are reported to undergo further degradation via different metabolic pathways. AMPA is the primary and major metabolite of the glyphosate degradation pathway [39], [43-44]. Among the intermediates, intracellular degradation of AMPA is non-feasibility making it a secondary source of glyphosate contamination in the environment [39], [43-44]. The relative comparison of threshold inhibitory concentration using EC<sub>50</sub> ranking of the single compound and their binary mixtures to the dehydrogenase activity of the isolates *Pseudomonas sp.* and *Bacillus sp.* demonstrated that DDVP was more toxic than glyphosate and the 20% Gly + 80% DDVP and 50% Gly + 50% DDVP ratios were the most toxic binary mixtures to the tested isolates. Furthermore, isobolographic characterization of the interactive effect of Glyphosate + DDVP binary mixtures indicated synergistic, antagonistic, and additive effects for different ratios of the mixtures against the test isolates. Glyphosate/DDVP mixtures ratios of 40% Gly/60% DDVP, 50% Gly/50% DDVP, 60% Gly/40% DDVP, and 80% Gly/20% DDVP against *Bacillus sp.* were all largely synergistic, while *Pseudomonas sp.* indicated synergistic, antagonistic, and additive effects for different ratios of the mixtures against the test isolates. Herbicides application poses a significant risk on microbial fauna depending on the application period [45]. Also, indirect risks to biodiversity occur due to the alternation in the physiological and biosynthetic mechanisms of soil ecosystems [46]. Some combinations of herbicides with heavy metals and inorganic fertilizers have been reported to inhibit the functions of microbial soil communities [47-48]. Such communities are highly intolerant of herbicides' synergistic interaction with other compounds also results [49]. The co-occurrence between toxicants may increase, decrease joint toxicity in soil or water. This joint toxicity could be stronger (synergistic), similar (additive), or weaker (antagonistic) than the single compound. Bioavailability and biotransformation of toxicants are important factors controlling toxicity of co-occurring toxicants [50].

#### 4. CONCLUSION

The present study investigated the toxicity of glyphosate and 2, 2-dichlorovinyl dimethyl phosphate binary mixtures on bacterial isolates from the Otamiri River. Our findings showed that

isolates exhibited tolerance to glyphosate and 2,2 dichlorovinyl dimethyl phosphate and their binary mixtures exposure at concentration range of 0-1000mg/L. However, binary mixtures of the pesticides beyond the hormetic range produced a mixture of interactions; majorly synergism, antagonism, and additivity were all obtainable. The nature of the interaction varied with the relative ratio of the component mixtures. A large hormetic range was mostly seen in the mixtures with a larger composition of glyphosate. It is evident that there are considerable differences in pesticide sensitivity among bacterial species and that the presence of glyphosate and 2, 2-dichlorovinyl dimethyl phosphate in the aquatic environment may present toxicological risk to microbial diversity.

#### 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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