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Types of Pesticides Interaction in Mixtures: Results of Inhibitory Assay

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Abstract—Enzymatic inhibitory assay based on the coupled enzyme system NAD(P)·H:FMN oxidoreductase and luciferase (Red + Luc), originally developed for environmental monitoring of soils, water, and air, is proposed as a method for evaluating changes in the properties of active ingredients of pesticide preparations depending on the additional components (formulants), as well as when pesticides are combined in mixtures. Using the commercial pesticide preparations containing glyphosate, it was shown that the degree of inhibition of the coupled enzyme system Red + Luc largely depends on the formulants rather than on the active ingredient in their composition. Moreover, the combined inhibitory effect of the pesticides mixture on the coupled enzyme system Red + Luc was not additive. According to the results of the inhibitory assay, the type of interaction of pesticide preparations in mixtures depends on both the formulants used and the ratio of pesticides in the mixture.

Keywords: pesticides, glyphosate, formulants, inhibitor analysis, luciferase, NAD(P)H:FMN oxidoreductase, bioluminescent analysis

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INTRODUCTION

Excessive repeated use of pesticides to control various pests and/or plant diseases has adverse impacts on both ecosystems and human health [1]. Moreover, to achieve a combined effectuse so-called “tank” mixtures of pesticides with a known individual toxic effect, but not a verified toxic effect of the pesticides mixture.

Currently, using various biological tests, it was demonstrated that active ingredients, as well as additives (formulants) in pesticide preparations, can interact with each other, which significantly changes their toxicological characteristics [2–4]. There is evidence that, when used together, pesticides either weaken (“antagonism”) or enhance (“sensitization”) each other’s effect. It was demonstrated that the effect of pesticide mixtures on *Daphnia*, algae, fish, and other test objects depends on the experimental conditions, on the ratio of concentrations of active ingredients of pesticides, and the properties of additional components in their composition [2–5]. The data obtained indicate the need for a comprehensive assessment of the used “tank” mixtures of pesticides, ensuring minimization of risks to consumer health.

To solve the problem of sustainable use of pesticides, inhibitor analysis based on the ability of toxic substances to reduce the activity of both individual enzymes and coupled enzymatic systems are very promising [6]. Such assays are characterized by rapidity, reproducibility of results, and simplicity, which generally allows for the analysis of a large number of samples in a short time.

Bioluminescent methods based on the coupled enzyme system of luminous bacteria NAD(P)·H:FMN oxidoreductase + luciferase (Red + Luc) have proven to work well for environmental monitoring of soil, water, and air, as well as for assessing the potential hazard of various substances (nanoparticles and nanomaterials, phenols and quinones, heavy metals, and pesticides) [6, 7]. In this paper, we assessed the prospects for using inhibitor analysis to determine changes in the characteristics of pesticides when they are used together in tank mixtures.

MATERIALS AND METHODS

In this work, the following reagents were used: FMN (Serva, Germany), NADH (Gerbu, Germany), tetradecanal (Merck, Germany), and lyophilized enzyme preparations produced by the Laboratory of

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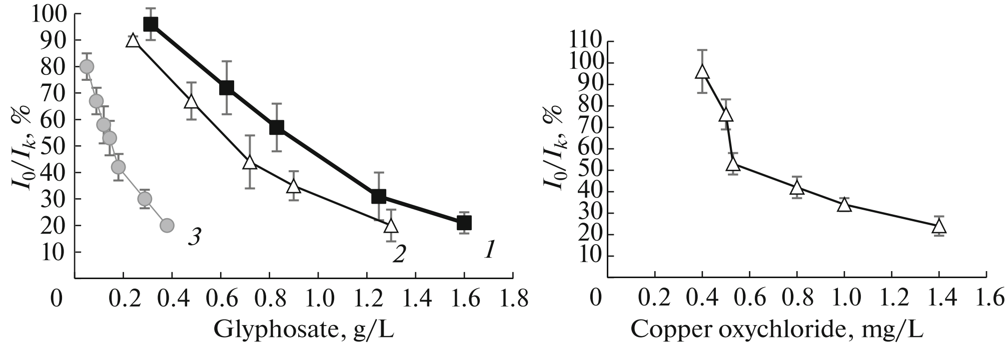

Fig. 1. The effects of commercial pesticide formulations on the coupled enzyme system Red + Luc. (a) The formulations with glyphosate as the active ingredient: *1*—"Tornado", *2*—"Glider", *3*—"Liquidator"; (b) The formulation "Abiga-Peak" with copper chloroxide as the active ingredient. Data are represented as $M \pm m$, $n = 5$.

Nanobiotechnology and Bioluminescence, Institute of Biophysics, Siberian branch of the Russian Academy of Sciences, Krasnoyarsk. One vial of the preparation contained 0.5 mg of luciferase (Luc, EC 1.14.14.3) from a recombinant *E. coli* strain and 3 nkat NAD(P)H:FMN oxidoreductase (Red, EC 1.5.1.29) from *Vibrio fischeri*. Enzyme solutions were prepared in 0.05 M potassium phosphate buffer (pH 7.0).

In this work, we assessed the inhibitory effect exerted on the coupled enzyme system Red + Luc by a mixture of the fungicide "Abiga-Peak"(Selkhozkhimiya, Russia), the active ingredient of which is copper oxychloride, and one of the herbicides in which glyphosate is used as the active ingredient (preparation "Liquidator" (Doctor Green, Poland), preparation "Glider" (Euro-seeds, Russia), and preparation "Tornado" (August, Russia)). Distilled water was used to prepare pesticide solutions.

The activity of the coupled enzyme system Red + Luc was determined by the value of the maximum luminescence intensity I_{max}, expressed in relative units. The influence of the analyzed samples on the activity of the enzymatic system Red + Luc was assessed by the residual luminescence $I_o/I_k \times 100\%$, where I_o and I_k are the values of the maximum luminescence intensity of the coupled enzyme system Red + Luc in the presence of the analyzed sample and the control solution, respectively. Distilled water was used as the control solution. The quantitative estimate of the degree of influence of pesticides on the activity of the coupled enzyme system Red + Luc was expressed as the values of IC_{20}, IC_{50}, and IC_{70}, which are the concentrations of the active ingredients of the pesticides that cause a decrease in the luminescence intensity of the coupled enzyme system Red + Luc by 20, 50, and 70%, respectively.

Table 1. IC_{20}, IC_{50}, and IC_{70} values (g/L) determined from the effects of commercial pesticide formulations on the coupled enzyme system Red + Luc

Active ingredient/ pesticide preparation	IC_{20}	IC_{50}	IC_{70}
Glyphosate/"Tornado"	0.55	1	1.25
Glyphosate/"Liquidator"	0.05	0.14	0.28
Glyphosate/"Glider"	0.32	0.68	1.05
Copper oxychloride/ "Abiga-Peak"	0.00047	0.00053	0.0011

RESULTS AND DISCUSSION

First, we plotted the dependences of the residual luminescence intensity of the coupled enzyme system Red + Luc on the concentrations of active ingredients in the composition of each of the pesticides (Fig. 1) and used them to determine IC_{20}, IC_{50}, and IC_{70} values (Table 1).

Figure 1a shows that three commercial pesticide preparations, the active ingredient of which is glyphosate, differ in their effect on the luminescence intensity of the coupled enzyme system Red + Luc. Among all the preparations, glyphosate in the preparation "Liquidator" had the greatest inhibitory effect: the IC_{50} value was 0.14 g/L. The degree of inhibition of the coupled enzyme system Red + Luc by the pesticides increased in the series "Tornado" < "Glider" < "Liquidator". The obtained results indicate the contribution of auxiliary components (formulants) in the composition of pesticide preparations to the overall inhibitory capacity, which is consistent with the results obtained by biotesting assays based on using living organisms [8]. Moreover, it was previously shown that the use of multienzyme systems for analysis often leads to an increase in the inhibitory effect of pesticides [9]. Thus, inhibitory assay is a promising method for comparative assessment of changes in the properties of pesticides depending on the formulas used.

Next, to analyze the effects of the mixture of pesticides on the coupled enzyme system Red + Luc, two pesticides in different ratios were simultaneously

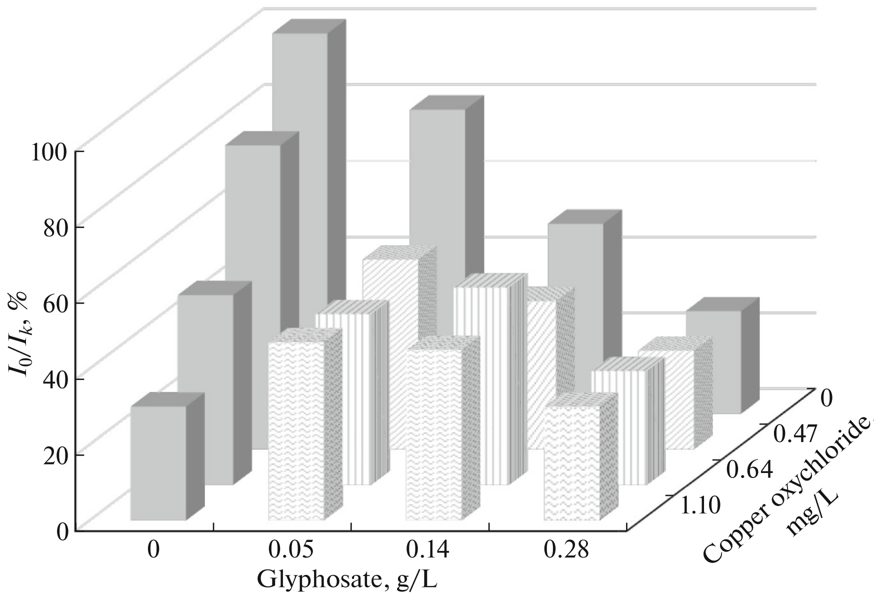

Fig. 2. Dependence of residual luminescence of the coupled enzyme system Red + Luc on the ratio of glyphosate (in the composition of the pesticide "Liquidator") and copper chloride (in the composition of the pesticide "Abiga-Peak") in the reaction mixture.

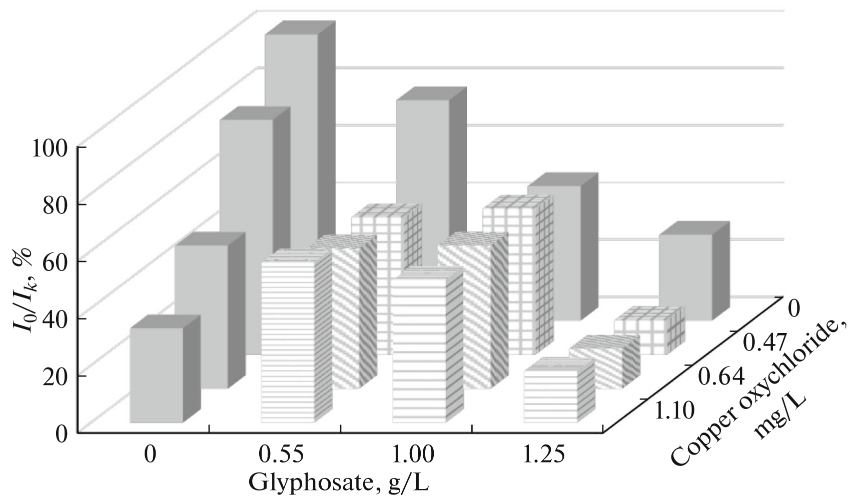

Fig. 3. Dependence of residual luminescence of the coupled enzyme system Red + Luc on the ratio of glyphosate (in the composition of the pesticide "Tornado") and copper chloride (in the composition of the pesticide "Abiga-Peak") in the reaction mixture.

added to the reaction mixture. Figures 2 and 3 show the effect of binary mixtures of pesticides "Liquidator" and "Abiga-Peak" and pesticides "Tornado" and "Abiga-Peak" on the coupled enzyme system.

The effect of the mixture of the pesticides "Liquidator" and "Abiga-Peak" on the coupled enzyme system Red + Luc was largely determined by the concentration of copper oxychloride in the pesticide "Abiga-Peak". For example, the effect on the coupled enzyme system exerted by the binary mixture containing copper oxychloride at a concentration of 1.1 mg/L (corresponds to the IC_{70} value) and different concentrations of glyphosate in the preparation "Liquidator" was classified as "antagonism;" i.e., the effect of the mixture was less than the effect of each of its components. With a decrease in the concentration of copper oxychloride, the effect exerted by the binary mixture was mainly classified as "synergism," because the effect of the mixture was more pronounced than the effect of each of its components taken separately, but less than

Table 2. Classification of the effects of a pesticide mixture on the luminescence intensity of the coupled enzyme system Red + Luc depending on the ratio of glyphosate and copper oxychloride in the composition of the pesticides “Liquidator” and “Abiga-Peak”, respectively

Copper oxychloride	Glyphosate		
	IC_{20}	IC_{50}	IC_{70}
IC_{20}	sensitization	synergism	synergism
IC_{50}	synergism	synergism	synergism
IC_{70}	antagonism	antagonism	antagonism

the sum of their effects (Fig. 2). The effect of the pesticide mixture containing minimum active concentrations of copper oxychloride and glyphosate, corresponding to their IC_{20}, was classified as “sensitization;” i.e., the effect of the mixture was greater than the effect of the sum of its components taken separately (Table 2). Similar results were obtained when analyzing the effect of the pesticide mixtures “Glider” and “Abiga-Peak” on the coupled enzyme system Red + Luc.

The effects of the “Tornado” and “Abiga-Peak” pesticide mixture on the coupled enzyme system Red + Luc varied greatly depending on the ratio of pesticides in the reaction mixture and included “antagonism”, “synergism”, “additivity,” and “sensitization” (Fig. 3).

There are two basic principles that describe how individual pesticides or additives can influence each other in a mixture: the concept of additivity (no influence) and the concept of interaction.

For a mixture of pesticides, additivity is observed when the pesticides have similar targets of action and their concentrations in the mixtures are significantly lower than the no observed adverse effect level (NOAEL) [10]. With increasing concentrations in mixtures, pesticides interact with each other, including toxicokinetic and toxicodynamic interactions. The interaction of pesticides when they are used together can lead to either the absence of the expected effect (in the case of interaction of the “antagonism” type) or a significant increase in the effect (in the case of interaction of the “sensitization” type). For example, the authors of [11, 12] showed that, in some cases, even pesticides with a similar chemical structure, when mixed, can have synergistic rather than additive effects. Numerous examples of the enhancement of the toxicity of pyrethroid, carbaryl, and triazine herbicides by organophosphates are known. Synergism between pyrethroid and carbamate compounds and antagonism between triazine herbicides and prochloraz are considered in the review [10].

In this work, inhibitory assay was used to demonstrate the absence of additivity in the effects of a pesticide mixture on enzyme functioning. Since the effect of pesticide mixtures containing glyphosate and copper oxychloride on the coupled enzyme system Red + Luc differed depending on the pesticide ratio, this indicates a dose-dependent interaction of the pesticides in the mixture, leading to a change in their inhibitory capacity.

In addition, using glyphosate as an example, we showed that pesticides with a single active ingredient interact differently with copper oxychloride, which again indicates that the properties of pesticides are largely determined by the formulants in their composition. The results obtained in this study demonstrate that, from the point of view of ecotoxicology and toxicology, formulants cannot be considered as inert substances. This conclusion is confirmed by many recent experimental studies [8, 13, 14].

Thus, the results of this work, obtained using inhibitory assay, are consistent with the results obtained by other biological tests. Changes in the effects of pesticides when they are mixed or when formulants are added are undesirable, and the consequences of using such mixtures are difficult to predict. Inhibitory assay is a rapid simple method that makes it possible, by their inhibitory capacity, to evaluate the properties of individual pesticides and changes in their properties when they are used in mixtures, as well as to determine the contribution of additives (formulants) to the final inhibitory effect of pesticide preparations on enzyme functioning.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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