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Monitoring of glyphosate-DNA interaction and synergistic genotoxic effect of glyphosate and 2,4-dichlorophenoxyacetic acid using an electrochemical biosensor☆

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ABSTRACT

Glyphosate (GLY) is a broad-spectrum herbicide used worldwide to control broadleaf sedge, and grass weeds to control non-specific vegetation. Although it was evaluated as non-toxic agent in 20th century, its carcinogenic and genotoxic potential has being intensively investigated all over the world in the last decade. Moreover, the combination of GLY and 2,4-dichlorophenoxyacetic acid (2,4-D) has been widely applied. Although genotoxicity of GLY has been evaluated *in vivo* studies, there is no report in the literature for the monitoring of *in vitro* biointeraction of GLY and double stranded DNA, or how effect the combination of GLY and 2,4-D onto DNA. Herein, an electrochemical biosensor platform was developed for detection of the pesticide-DNA interaction by using disposable pencil graphite electrodes (PGEs). First, voltammetric detection of the interaction between GLY and DNA was investigated and the electrochemical characterization of the interaction was achieved. Taking a step further, the synergistic genotoxic effect of the mixture of GLY and 2,4-dichlorophenoxyacetic acid (2,4-D) or the mixture of their herbicide forms onto DNA could be monitored. This effect was concentration dependent, and the herbicide of GLY or the use of mixture of herbicides of GLY and 2,4-D had more genotoxic effect than analytical grade of the active molecules, GLY and 2,4-D. The single-use PGEs provided to fabricate robust, eco-friendly and time saver recognition platform for monitoring of herbicide-DNA interaction with the sensitive and reliable results. It is expected that this study will lead to be designed miniaturized lab-on-a chip platforms for on-line analysis of the pesticide-nucleic acid interactions.

1. Introduction

Glyphosate (GLY) (N-phosphonomethyl glycine) is an active ingredient widely used as plant protection products since 1971 (Duke, 2017; Muñoz et al., 2020; Nagya et al., 2019). Although World Health Organization reported that GLY possess very low toxicity in 1997 (Carvalho et al., 2020), continued studies revealed that GLY has genotoxic (Carvalho et al., 2020; Guyton et al., 2015; Nagya et al., 2019) and cytotoxic effects (Toth et al., 2020; Trasande et al., 2020). Furthermore, GLY was classified as "probably carcinogenic to humans" (Group 2A) in 2015 by The International Agency for Research on Cancer (IARC 2015). It was reported that GLY had genotoxic potential in human mononuclear white blood (HMWB) cells (Nagya et al., 2019), caused endocrine disruption in rats (Nardi et al., 2017), affected to nervous system in humans (Rueda-Ruzafa et al., 2019) and oocyte quality in mouses (Yahfoufi et al., 2020). Therefore, the use of GLY based herbicide formulations has been debated by countries. Turkey banned to use of Roundup which is a GLY based and widely used herbicide formulation worldwide. It contaminates water sources, soil and even air by transporting as particles (Valle et al., 2019). Thus, to enlighten the genotoxic effect of GLY has gained importance. There are lots of *in vivo* (Carvalho et al., 2020) and *in vitro* (Nagya et al., 2019; Silva et al., 2020; Zhang et al., 2019) studies in the literature to evaluate the genotoxic effect of GLY. It is reported that GLY caused DNA double strand-breaks (Woźniak et al., 2018; Zhang et al., 2019).

2,4-dichlorophenoxyacetic acid (2,4-D) is an auxinic herbicide widely used to control broadleaf weeds and plants (Arcaute et al., 2016). It binds to double strand DNA and caused DNA damage

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and strand-breaks (Ahmadi and Bakhshandeh, 2009; Arcaute et al., 2016). GLY and 2,4-D are the herbicides used for large crop production. It is possible to use them together (Carvalho et al., 2020; Robinson et al., 2012). Carvalho and co-workers reported that the combination of GLY plus 2,4-D or GLY plus 2,4-D-amine showed a synergistic pattern by means of genotoxic effect of GLY and 2,4-D (Carvalho et al., 2020).

In vitro analysis of the interaction of (bio)molecules and double-stranded DNA has been investigated by using electrochemical biosensors since the discovery of electroactive structure of nucleic acids (Palecek, 2015; Palecek and Bartošík, 2012; Palecek and Dorcák, 2017). Lots of biomolecules (Carvalho et al., 2019; Erdem et al., 2011; Hermanová et al., 2019; Ilkhani et al., 2016; Morawska et al., 2018; Tersch and Lisdat, 2011; Zhang et al., 2018) have been studied in this field. The changes at the electrochemical responses obtained before/after interaction between DNA and the (bio)molecule are evaluated. The electrochemical biosensors for monitoring of the biomolecule-nucleic acid interaction offer to develop cheap, practical, time-saving, easy-handling tools for diagnosis of the interaction process, or enlighten how the (bio) molecule affects DNA structure (Kogikoski et al., 2019; Lima et al., 2020; Lu, 2018; Palecek and Bartošík, 2012). Thus, the bio-interaction of the pesticides and nucleic acids is an attractive topic in the field of electrochemical diagnosis and researchers studied by different types of pesticides for this purpose. As an example, Lima et al. (2010) reported an electrochemical biosensor for monitoring of DNA damage caused by sulfentrazone. They used glassy carbon electrode as immobilization surface for double stranded (ds) and single stranded (ss) DNA. First, GCE was polished and cleaned, then, multiwalled carbon nanotube (MWCNT) was modified at the surface of GCE. Ten mg/mL dsDNA or 3 mg/mL ssDNA was immobilized at the surface of MWCNT/GCE, then the interaction was performed by inserting the electrode in sulfentrazone solution. But MWCNT/GCE was not single-use, required complicated cleaning and modification steps and required the use of quite high concentrations of ssDNA and dsDNA.

Ahmadi et al. (2011) investigated chloridazon and DNA interaction by using hanging mercury drop electrode (HMDE) and GCE. They implemented cathodic and anodic differential pulse voltammetric measurements by using HMDE and GCE, respectively. Although they found how chloridazon affected double stranded DNA, neither HMDE nor GCE had miniaturizable form, their preparation required long time and labor-intensive method and HMDE had toxic effects due to the use of mercury.

MWCNT and chitosan (CHIT) modified PGEs were developed for detection of amitrole based on the biointeraction principle between dsDNA and herbicide (Ensafi et al., 2013). The PGEs were coated with teflon band, polished, then modified with MWCNT/CHIT nanocomposite. One mg/mL dsDNA was immobilized at the electrode surface. dsDNA immobilized MWCNT/CHIT-PGEs were immersed into the amitrole solution and depositing was performed by stirring the amitrole solution in open circuit system. Although PGEs were used in this report, the preparation of them were quite complicated and time-consuming. Also, the method reported in the study required high DNA concentration and sample volume.

Electrochemical detection platforms developed for detection GLY were reported in the literature (Bochkova et al., 2020; Cahuantzi-Muñoz et al., 2019; Gholivand et al., 2018; Regiarti et al., 2020; Sok and Fragoso, 2019; Vaghela et al., 2018; Zouaou et al., 2020), but there is no report for development of the electrochemical biosensor to monitor GLY-DNA interaction. Herein, it is aimed to bring about a new application in the field of genotoxicity assessment of the herbicides by using a disposable electrochemical biosensor. For this purpose, (i) interaction occurred between GLY and double stranded DNA and (ii) synergistic genotoxic effect of GLY and 2,4-D by means of DNA damage using not only their ingredients but also their herbicide forms was monitored by using disposable pencil graphite electrodes (PGEs). First, DNA immobilized at the surface of PGEs, then, the surface-confined interaction in the presence of GLY was done. The oxidation signal of guanine was measured by using differential pulse voltammetry (DPV) technique and the decrease at the guanine signal was evaluated by means of the interaction. The electrochemical characterization of the interaction was performed using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. This is the first study in the literature reported for the purpose of investigation GLY and DNA interaction and monitoring the synergistic effect of GLY and 2,4-D by means of DNA damage using disposable electrochemical biosensor.

2. Material and methods

2.1. Apparatus

IVIUM Compactstat.e with IVIUM Release 4.951 software package (Holland) was used for all electrochemical measurements.

Three electrode system was formed using pencil graphite electrode (PGE) as working electrode, an Ag/AgCl/3M KCl as reference electrode (BAS, Model RE-5B, W. Lafayette, USA) and a platinum wire as the auxiliary electrode. The graphite lead (Tombow 0.5 HB, Japan) was gripped by Rotring Pencil model (Germany). The electrical contact between the lead and the pencil was achieved by soldering a metallic wire to the metallic part. Forteen-mm of the lead was held and 10 mm of the lead was immersed into the solution during each measurement.

2.2. Chemicals

Double stranded fish sperm DNA (DNA), glyphosate (GLY) and 2,4-dichlorophenoxyacetic acid (2,4-D) were purchased from Sigma-Aldrich. DNA stock solution was prepared at 1000 μg/mL concentration level in Tris-EDTA buffer solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.00) and kept frozen. DNA was diluted in 0.50 M acetate buffer containing 20 mM NaCl (pH 4.80, ABS). GLY or 2,4-D stock solutions at 1000 μg/mL were prepared using 50 mM phosphate buffer solution (PBS; pH 7.40) (Gholivand et al., 2018) or DMSO, respectively. The diluted solutions of 2,4-D and GLY were prepared with 50 mM PBS (pH 7.40) (Gholivand et al., 2018; Navratilova and Skladal, 2004).

Herbicides whose formulation are GLY potassium salt and ether amine ethoxylate (herbicide-1) or 2,4-D amine salt (herbicide-2) were purchased from local market. The stock concentrations of herbicide-1 and herbicide-2 were 441.000 and 500.000 μg/mL, respectively. The diluted solutions of the herbicides were prepared in PBS (pH 7.40).

Other chemicals were of analytical reagent grade and were supplied from Sigma-Aldrich and Merck. Ultrapure water was used in other stock solutions.

2.3. Procedure

2.3.1. Preparation of DNA immobilized PGE

PGEs were electrochemically pretreated by applying a potential of +1.40 V for 30 s in ABS (pH 4.80). Passive adsorption was used to immobilize DNA at the surface of PGE. For this purpose, 100 μL of 30 μg/mL DNA was immobilized by dipping the electrodes at the surface of PGE during 15 min. DNA immobilized PGEs were then washed with ABS (pH 4.80) to eliminate unspecific binding.

2.3.2. The interaction of DNA and GLY at the surface of PGE

Thirty μg/mL DNA immobilized electrodes were immersed in 100 μL of 25–150 μg/mL GLY at dark during 5–30 min. Then, all electrodes were washed with PBS (pH 7.40).

2.3.3. The interaction of DNA and 2,4-D, the herbicides, the mixture of GLY:2,4-D or the mixture of the herbicides

For the interaction in the presence of 2,4-D, DNA immobilized PGEs were immersed into the vials containing 100 μL of 25–75 μg/mL 2,4-D.

For the interaction in the presence of the mixtures of GLY:2,4-D, DNA immobilized electrodes were dipped into the vials containing 100: 25–75 μg/mL GLY:2,4-D samples.

For the interaction of DNA and herbicide-1 or herbicide-2, DNA immobilized PGEs were immersed into the vials containing 100 μL of 5–25 μg/mL herbicide-1 or 10–40 μg/mL herbicide-2 sample.

For the interaction in the presence of the mixtures of herbicide-1:herbicide-2, DNA immobilized electrodes were dipped into the vials containing 25 μg/mL herbicide-1: 10–40 μg/mL:herbicide-2 samples. Five min interaction time was chosen for the interaction studies performed in this section based on the results obtained in section 2.3.2.

After all immobilization steps, the electrodes were washed in PBS (pH 7.40). All interaction steps were performed at dark.

2.3.4. Voltammetric measurements

Before/after interaction process, the oxidation signal of guanine was measured at +1.025 V and the changes at the guanine signal were evaluated in terms of the interaction. For this purpose, differential pulse voltammetry (DPV) technique was used (Congur et al., 2021). DPV measurements were performed in ABS (pH 4.80) between the potential of +0.75 V and +1.25 V at a pulse amplitude of 50 mV and scan rate of 50 mV/s. The raw data obtained after the interaction of DNA and GLY or DNA and herbicides were treated by the Savitzky and Golay filter 11 points or 5 points, respectively. Average baseline was then implemented for all datas.

Cylic voltammetry (CV) measurements were performed in 2.00 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) prepared in 0.10M KCl using the potential range from −0.45 V to +1.20 V with the scan rate as 50 mV/s (Congur et al., 2021).

2.3.5. Impedimetric measurements

Impedimetric measurements were performed in 2.50 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) prepared in 0.10 M KCl. The impedance was measured in the frequency range from 100 mHz to 100 kHz at a potential of +0.23 V with a sinusoidal signal of 10 mV. The frequency interval divided into 98 logarithmically equidistant measure points. The data was fitted by Randles circuit. Charge transfer resistance (R_{ct}), solutions resistance (R_s), the capacitance (Q), and Warburg impedance (W) are the elements of the circuit. R_{ct} represents the respective semicircle diameter and the changes at the R_{ct} value were evaluated in terms of interaction process (Congur et al., 2021; Erdem et al., 2011).

3. Results and discussion

At the first step of the study, the effect of GLY concentration upon the interaction process was investigated. The changes at the guanine signal were evaluated for the effect of concentration of GLY on interaction process. Before interaction, the average guanine signal was obtained as 4.98 μA with the relative standard deviation % (RSD%) as 9.78% (n = 3) (Fig. 1B–a). After interaction, decrease at the guanine signal was observed due to the strand-break effect of GLY (Zhang et al., 2019; Woźniak et al., 2018). There were 8.68%,

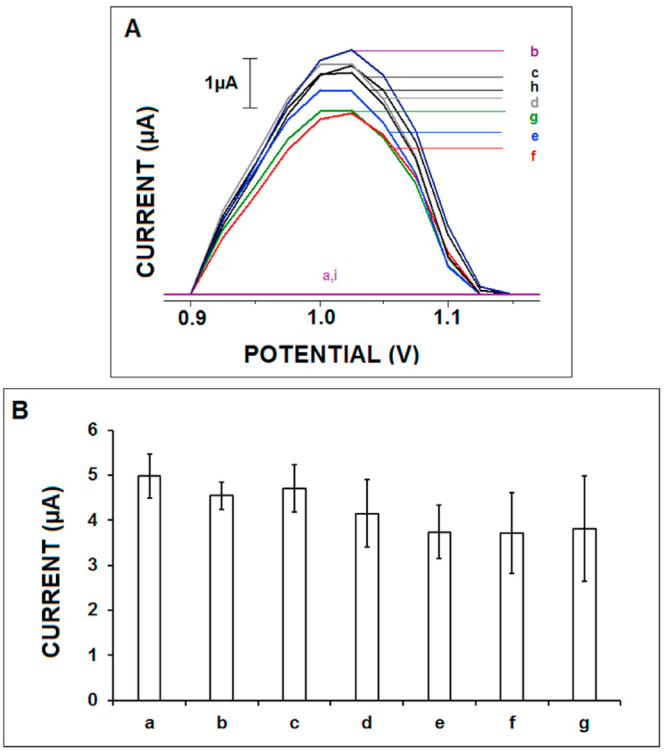

Fig. 1. Voltammograms **(A)** of the guanine signals obtained before and after the interaction of 30 μg/mL DNA and 25–150 μg/mL GLY at PGE surface. Control signal of PGE **(a),** DNA immobilized PGE **(b),** the interaction between 25 **(c),** 50 **(d),** 75 **(e),** 100 **(f),** 125 **(g)** and 150 **(h)** μg/mL GLY and 30 μg/mL DNA at the surface of PGE, control signal of GLY **(i).** Histograms **(B)** of the average guanine signals obtained before **(a)** and after 25 **(b),** 50 **(c),** 75 **(d),** 100 **(e),** 125 **(f)** and 150 **(g)** μg/mL GLY and 30 μg/mL DNA at the surface of PGE (n = 3). Measurements were performed using DPV technique.

5.26%, 16.58%, 24.94%, 25.26%, 23.44% decrease at the guanine signal after the interaction of 30 μg/mL DNA and 25–150 μg/mL GLY, respectively. The highest decrease at the guanine signal could be obtained at 100 μg/mL concentration level of GLY (Fig. 1B–e) as 24.94% with the reproducible results (RSD% = 15.96% (n = 3)) and the average guanine signal was measured as 3.74 ± 0.6 μA. Thus, 100 μg/mL concentration level of GLY was chosen for the investigation of the effect of interaction time upon the biosensor response (Fig. S1). The interaction was performed between DNA and 100 μg/mL GLY during 5,15 or 30 min. The highest decrease at the guanine signal could be estimated as 24.94% (Fig. S1B-a to b) after 5 min interaction; therefore, this interaction time was used in the next experimental steps.

The electrochemical characterization of the interaction between DNA and GLY at the surface of PGE was investigated by using CV and EIS techniques. Fig. S2 represents the voltammograms and the average cathodic peak current (I_c) values obtained by CV technique. The average I_c could be obtained by using PGEs as 79.17 μA with the RSD% = 4.48% (n = 3) (Figs. S2B–a). After DNA immobilization, the average I_c decreased due to the repulsive interaction between negatively charged phosphate backbone of double-stranded DNA and anionic redox probe (Prasad and Fatma, 2017). Decrease ratio at I_c was found to be 28.30%. After the interaction of DNA and 100 μg/mL GLY, the I_c also decreased (Figs. S2B–c). This decrease could be attributed the increase of the negative characteristic of the electrode surface by introducing of GLY molecules after interaction process as well as DNA breakage. GLY is a zwitterionic pollutant which has net dianion at pH > 6 (Ehrl et al., 2018). The I_c value decreased after the interaction of DNA and GLY due to formation of

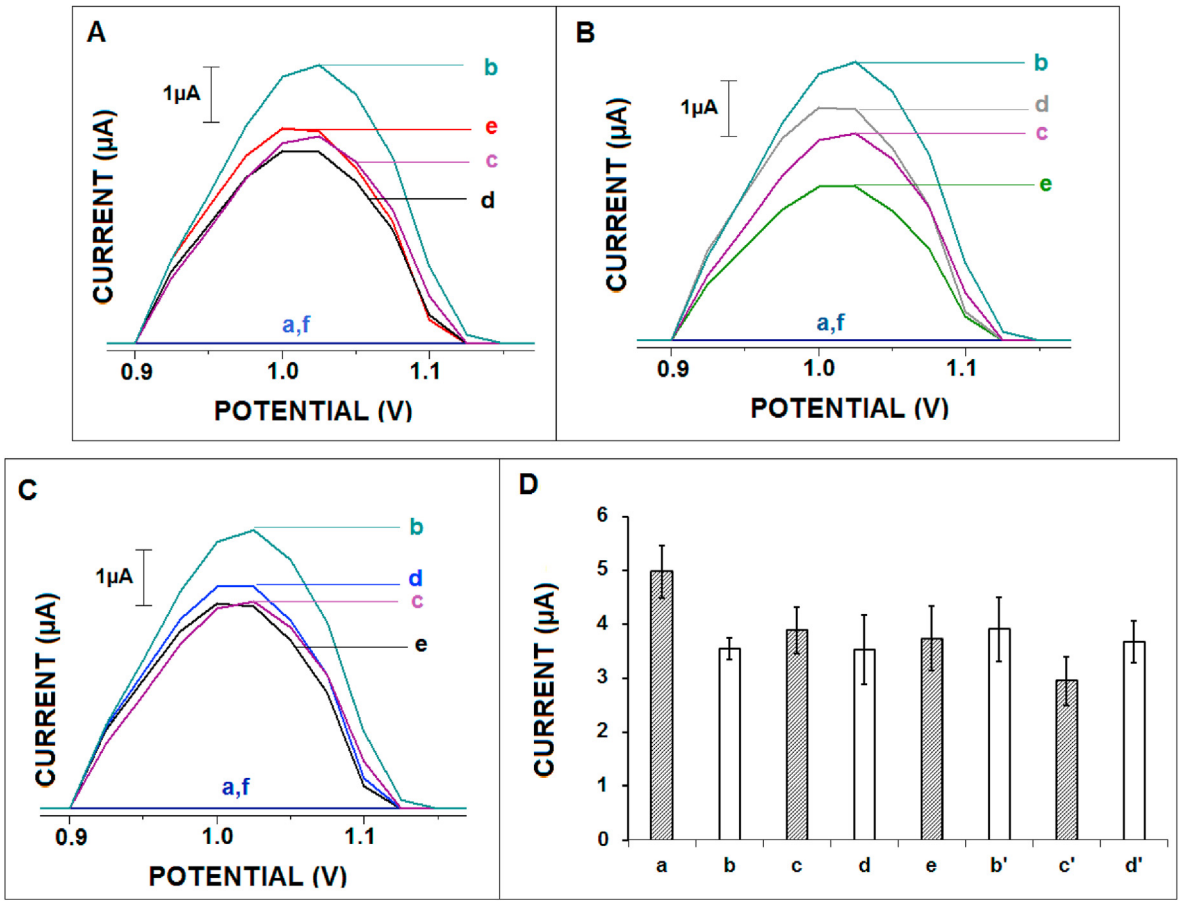

Fig. 2. Voltammograms representing the guanine signals obtained after the interaction of 100 μg/mL GLY, 2,4-D or the mixture of GLY:2,4-D at different concentration level of 2,4-D as 25 **(A),** 50 **(B)** and 75 **(C)** μg/mL. Control signal of PGE **(a),** guanine signal obtained by 30 μg/mL DNA immobilized PGE **(b),** the guanine signals obtained after the interaction of 30 μg/mL DNA and 100 μg/mL GLY **(c),** 25–75 μg/mL 2,4-D **(d)** and the mixture of 100 μg/mL GLY: 25–100 μg/mL 2,4-D (1:1) at the surface of PGE. Histograms **(D)** representing the average guanine signals (n = 3) obtained by 30 μg/mL DNA immobilized PGE **(a),** after the interaction of 30 μg/mL DNA and 25 (**b**), 50 **(c),** 75 (d) μg/mL 2,4-D or 100 μg/mL GLY **(e),** after the interaction of 30 μg/mL DNA and the mixture of 25 **(b'),** 50 **(c'),** 75 **(d')** μg/mL 2,4-D and 100 μg/mL GLY at the surface of PGE.

repulsive forces between the redox probe and the electrode surface. The decrease ratio at I_c was calculated as 30.85%.

The cathodic charge values (Q_c) were also measured during CV measurements for each immobilization/interaction step. The average Q_c obtained by PGE could be obtained as 4.43×10^{-4} C (RSD % = 3.36%, n = 3) whereas the average Q_c obtained by DNA immobilized PGE was 3.96×10^{-4} mC (RSD% = 3.21%, n = 3). After the interaction of GLY and DNA, the average Q_c was measured as 3.07×10^{-4} C (RSD% = 4.61%, n = 3). As clearly seen, the average Q_c decreased after DNA immobilization or the interaction process as expected. The results based on the average Q_c values were consistent with the results based on the average I_c values.

A_{eff} values were calculated according to the Randles–Sevcik Eq (Eq. (1)) (Cummings and Elving, 1978) using I_C values. The transferred electron number is n, D is the diffusion coefficient of $K_4[Fe(CN)_6]$ (7.6×10^{-6} cm^2 s^{-1}), C is the concentration of $K_4[Fe(CN)_6]$ in this equation.

$$i_p = 2.69\ 10^5 n^{3/2} A_{eff} D^{1/2}\ C\ v^{1/2} \quad (1)$$

The A_{eff} values obtained by PGE, DNA immobilized PGE, after the interaction of DNA and GLY were found to be 0.239, 0.171 and 0.118 cm^2, respectively.

The peak-to-peak separation values (ΔE_p) were also calculated for PGE, DNA immobilized PGE, after the interaction of DNA and GLY at the surface of PGE as, 230, 330 and 410 mV, respectively. These results were indicated that the DNA immobilization and the interaction provided that the electrochemical reaction of $K_4[Fe(CN)_6]$ ions onto the PGE surface was irreversible.

Fig. S3 represents the Nyquist diagrams (A) and the histograms (B) of the average R_{ct} values obtained by EIS. The average R_{ct} could be measured as 82.50 Ohm with the RSD% = 2.57% (n = 3). After DNA immobilization, 19.36 fold increased R_{ct} was obtained (Fig. S3A,B-a to b). This increase could be attributed that the repulsive interaction of negatively charged redox probe and DNA molecules (Erdem and Congur, 2013). After the interaction of GLY and DNA, the electrode surface became more negative by introducing negatively charged GLY molecules (Ehrl et al., 2018). Thus, the interaction was resulted the increase at R_{ct} (Fig. S3A,B-b to c).

The apparent fractional coverage (Q_R^{IS}) values were calculated for DNA immobilization and the interaction process based on the R_{ct} values obtained before/after each immobilization/interaction step (Eq. (2)) (Erdem and Congur, 2013; Janek et al., 1998) and found to be 0.95 and 0.38, respectively. These results showed the immobilization of DNA and the interaction of GLY and DNA at the surface of PGE could be successively performed.

$$Q_{IS}^{R} = 1 - R_{ct1} \Big/ R_{ct2} \quad (2)$$

The voltammetric and impedimetric results were consistent with each other and showed that the surface-confined interaction of DNA and GLY could be succesfully achieved under optimum

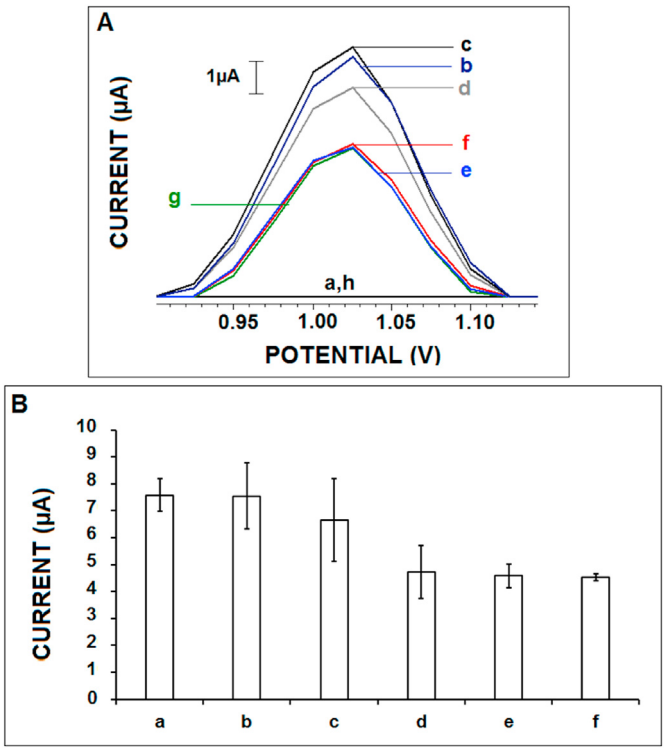

Fig. 3. Voltammograms **(A)** of control signal of PGE **(a),** the guanine signal obtained by 30 μg/mL DNA immobilized PGE **(b),** after the interaction of 30 μg/mL DNA and 5 **(c),** 10 **(d),** 15 **(e),** 20 **(f)** and 25 **(g)** μg/mL herbicide-1. Histograms **(B)** of the average guanine signals obtained by 30 μg/mL DNA immobilized PGE **(a),** after the interaction between 30 μg/mL DNA and 5 **(c),** 10 **(d),** 15 **(e),** 20 **(f)** and 25 **(g)** μg/mL herbicide-1 at the surface of PGE (n = 3).

conditions.

In the next step of the present study, the synergistic effect of GLY and 2,4-D onto DNA was investigated. For this purpose, the interaction between mixture of 100: 25–75 μg/mL GLY:2,4-D and DNA was performed at the surface of PGE (Fig. 2). After the interaction of DNA and GLY, the guanine signal decreased as 24.94% and the average guanine signal was found to be 3.73 μA with the RSD % = 15.96% (n = 3) (Fig. 2D–a to e). The same behaviour at the guanine signal was observed after the interaction of 2,4-D and DNA due to the strand break effect of 2,4-D (Ahmadi and Bakhshandeh, 2009; Arcaute et al., 2016). After the interaction of 25, 50 and 75 μg/mL 2,4-D and DNA, the guanine signal decreased and the decrease ratios were calculated as 28.65%, 26.57% and 29.15%, respectively. The synergistic effect of the mixture of GLY and 2,4-D could be observed at 50 μg/mL concentration level of 2,4-D (Fig. 2B). The most decrease at the guanine signal could be monitored in the presence of the mixture of 100:50 μg/mL GLY:2,4-D (Fig. 2B–b to e). The decrease ratio was found to be 40.71% and the average guanine signal was estimated as 2.95 μA (RSD% = 15.15%, n = 3) (Fig. 2D-c'). Higher decrease at the guanine signal could be observed after the interaction of the mixture of 100 μg/mL GLY and 50 μg/mL 2,4-D than the ones obtained in the presence of GLY or 2,4-D individually which indicated the synergistic effect of the use of mixture of GLY and 2,4-D onto DNA. The results were in a good agreement with the results reported by Carvalho et al. (2020).

In the second part of the study, the interaction of the herbicide whose active molecule was GLY (herbicide-1) and DNA was investigated at the surface of PGE. Five points smooth level was applied onto the raw data due to the oxidation signal of herbicide measured at 0.850 V and the guanine signal measured at +1.025 V was overlapped using 11 points smooth level (Fig. S4). The interaction of DNA and 5–25 μg/mL herbicide-1 was performed during 5 min (Fig. 3). The guanine signal sharply decreased at 15 μg/mL concentration level of herbicide-1. Although the decrease was limited at higher concentrations, the most reproducible signal could be achieved at 25 μg/mL concentration level of herbicide-1 as 4.53 μA (RSD% = 3.14%, n = 3). Twenty-five μg/mL herbicide-1 was chosen as optimum for the next study.

The synergistic effect of the mixture of the herbicide-1 and herbicide-2 onto DNA was investigated in the last part of study (Fig. 4). The concentration-dependent effect of 2,4-D was also observed by using herbicide-2 sample. The decrease at the guanine signal was monitored at each concentration level of herbicide-2, but the highest decrease at the guanine signal could be monitored in the presence of 25:20 μg/mL herbicide-1:herbicide-2 (Fig. 4E–a to c') and the decrease ratio and the average guanine signal was found to be 47.45% and 3.46 μA (RSD% = 6.66%, n = 3), respectively. Carvalho et al. (2020) reported that GLY and 2,4-D or GLY and the amine form of 2,4-D had synergistic pattern. The voltammetric results obtained after the interaction between DNA and the mixture of the herbicides were consistent with the literature. But the synergistic effect could be monitored at lower concentrations of the herbicides than the one obtained in the presence of GLY and 2,4-D.

High concentrations of GLY and 2,4-D is used in the herbicide as stock solution. Although the concentration is depending on the implementation, the lowest suggested concentrations in the user manuals equals to 33075 and 20000 μg/mL per hectare for the herbicides of GLY and 2,4-D, respectively. These concentration levels are quite higher than the concentration levels at which the synergistic effect was observed for both GLY and 2,4-D and the herbicides of GLY and 2,4-D. On the other hand, it is possible to use of the herbicides at higher concentrations than the ones stated in the user manual by farmers or agricultural laborers due to lack of agricultural education.

Daam et al. investigated the lethal toxicity of GLY to tropical frog larvae (Daam et al., 2019). They found that 96 h-LC_{50} as 115 and 106 μg/mL for *Physalaemus cuvieri* and *Hypsiboas pardalis*, respectively by using analytical grade of GLY. In the present study, the highest decrease at the guanine signal was shown after the interaction of 100 μg/mL GLY and 30 μg/mL double stranded DNA extracted from salmon sperm. The results were in an agreement than the results reported by Daam et al.

In the study reported by Zhang and coworkers, 500 μM GLY (equals to 84.5 μg/mL) was used to determine the effect of GLY onto mouse oocytes (Zhang et al., 2019). It was reported that GLY had cytotoxic and genotoxic effect on mouse oocytes at this concentration level and caused double strand DNA break. In the present study, the decrease at the guanine signal could be monitored in the presence of 25–150 μg/mL GLY (equals to 147.87–887.2 μM). Therefore, the results represented in this current report are parallel with the results given in the literature.

Alvarez-Moya et al. (2014) investigated the genotoxicity of GLY on plants, humans and animals. They used 0.0007–0.7 mM (equals to 0.12–118.35 μg/mL) concentration range. They found that the increase in DNA migration was significant after the exposure of different concentration of the herbicide of GLY. They also implemented the comet assay studies using GLY. They compared the genotoxicity of GLY onto human lymphocytes, fish erythrocytes and plant stamen nuclei *in vivo* and *in vitro*. Although the effect was concentration-dependent, they found that GLY has genotoxic effect for plants, humans and animals. The concentration range reported in that study includes the concentration range used in the present study for GLY (25–150 μg/mL) and the herbicide of GLY (5–25 μg/mL). Therefore, the results represented herein are in a good

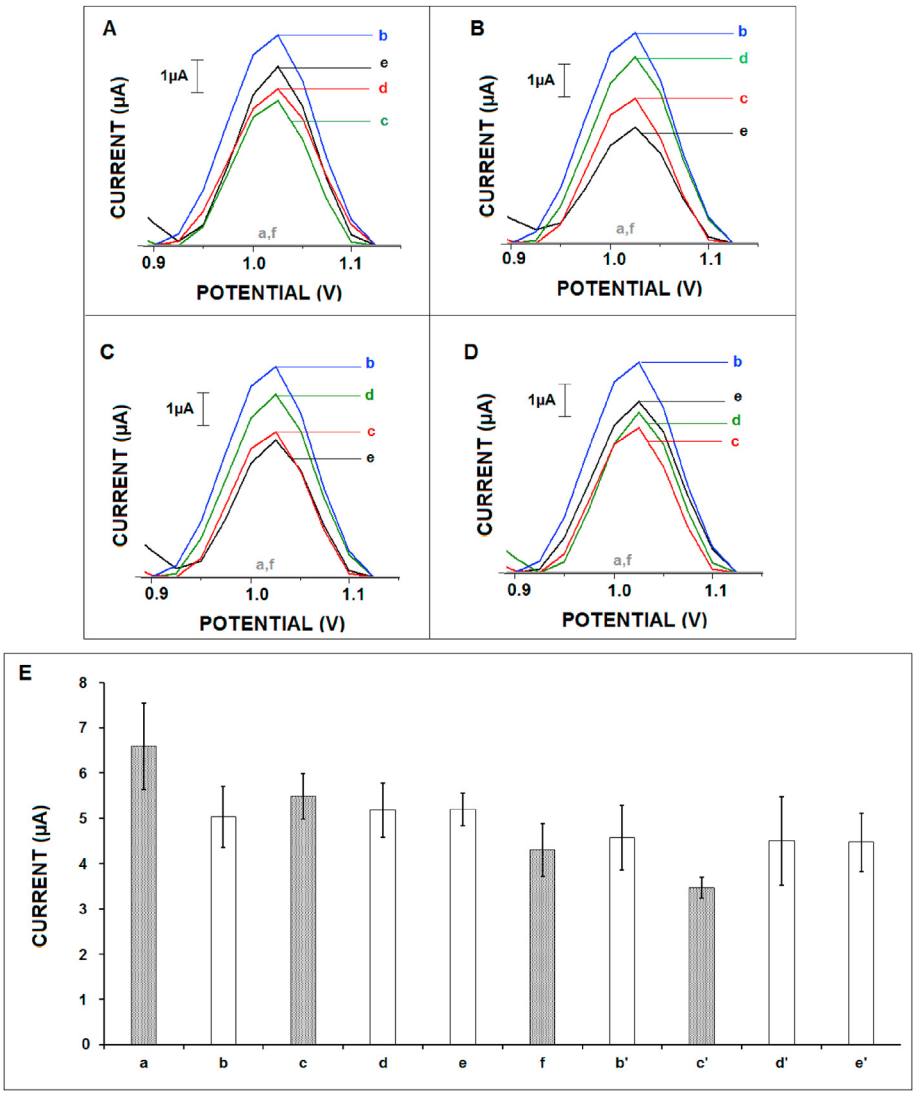

Fig. 4. Voltammograms representing the guanine signals obtained after the interaction of 25 μg/mL herbicide-1, herbicide-2 or the mixture of herbicide-1:herbicide-2 (1:1) at different concentration level of herbicide-2 as 10 **(A),** 20 **(B),** 30 **(C)** and 40 **(D)** μg/mL. Control signal of PGE **(a),** guanine signal obtained by 30 μg/mL DNA immobilized PGE **(b),** the guanine signals obtained after the interaction of 30 μg/mL DNA and 25 μg/mL herbicide-1 **(c),** 10–40 μg/mL herbicide-2 **(d)** and the mixture of 25 μg/mL herbicide-1: 10–40 μg/mL herbicide-2 (1:1) **(e)** at the surface of PGE. Histograms **(E)** representing the average guanine signals (n = 3) obtained by 30 μg/mL DNA immobilized PGE **(a),** after the interaction of 30 μg/mL DNA and 10 **(b),** 20 **(c)**, 30 **(d)** and 40 μg/mL **(e)** herbicide-2 or 25 μg/mL herbicide-1 **(f),** after the interaction of 30 μg/mL DNA and the mixture of 10 **(b'),** 20 **(c'),** 30 **(d')** and 40 **(e')** μg/mL herbicide-2 and 25 μg/mL herbicide-1 (1:1) at the surface of PGE.

agreement with the literature.

Carvalho et al. (2020) implemented single cell gel electrophoresis (SGCE) assay to show synergistic genotoxic effect of the mixture of GLY and 2,4-D. They exposured the mixture of GLY and 2,4-D to fishes during 96 h, then collected blood cells and analysed cell lysates.

Considering all these reports given above (Alvarez-Moya et al., 2014; Carvalho et al., 2020; Daam et al., 2019; Woźniak et al., 2018; Zhang et al., 2019) reported for investigation of GLY genotoxicity, all of them include *in vitro and in vivo* experiments. These *in vivo* studies require the use of plant and animal experiments, or the use of complex chemical agents which is resulted environmental burden. All these experimental steps are labor-intensive and time-consuming, their implementation takes days. On the other hand, the proposed biosensor offers to monitor the genotoxicity caused by the herbicides in just 20.5 min (20 min preparation, 0.5 min measurement) with the reliable results. The electrochemical biosensor represents another advantage by having eco-friendly structure due to the fact that PGEs require just 100 μL sample of DNA or the herbicides. Moreover, not only herbicide genotoxicity but also the genotoxic effect of herbicide-herbicide interaction could be monitored by using developed electrochemical biosensor as the first time in the literature. The biosensor platform is appropriate to fabricate hand-held devices for monitoring of the genotoxic effects of different types of herbicides, implementation of the biosensor is not limited with GLY. Single-use electrochemical recognition platform developed in this study will be preferable in this area due to the fact that it eliminates all drawbacks of conventional techniques and also it presents consistent results with the literature.

Although *in vitro* detection of DNA damage caused by pesticides using electrochemical methods were introduced in the literature by different groups (Ahmadi et al., 2011; Ensafi et al., 2013; Lima et al., 2010), none of them evaluated the effect of the herbicides, they only includes the electrochemical investigation of the analytical grade of active molecules. It should be pointed out that another important

result obtained within the scope of the study could be represented, the herbicide form of GLY caused the decrease at the guanine signal at lower concentration level than GLY. This results indicated that the herbicide form of GLY has more genotoxic effect than analytical grade of GLY which is parallel with the results reported in the literature (Peillex and Pelletier, 2020; Woźniak et al., 2018). Also, the combination of GLY and 2,4-D had more genotoxic effect than the use each of them individually, but the combination of herbicides of GLY and 2,4-D had this genotoxic effect at lower concentrations, e.g., 25:20 μg/mL herbicide of GLY: herbicide of 2,4-D.

4. Conclusion

Herein, a robust, sensitive, practical, time-saving and eco-friendly electrochemical biosensor platform was developed for monitoring of the genotoxic effect of GLY and synergistic pattern of GLY and 2,4-D as the first time in the literature. It can be concluded that GLY has negative effect onto double stranded DNA by using it alone or in combination with 2,4-D. This genotoxic effect could be monitored at lower concentrations of the herbicides of GLY and 2,4-D which means that the herbicide forms of them have more side effect. The obtained results showed the consistency of the study with conventional studies reported in the literature, but the genotoxicity of the herbicides could be determined using fewer chemical agents without animal trials, or complex experimental steps.

It's expected that this study will lead to further investigations carried out for the purpose of understanding of the pesticide-DNA interaction or the effect of pesticide-pesticide interaction onto DNA using electrochemical biosensors. This study will bring into a new aspect for *in vitro* determination of genotoxicity caused by not only pesticides but also different environmental hazardous materials. The principles presented in this study for monitoring of pesticide-DNA interaction will be pioneering for the fabrication of the miniaturized lab-on-a chip systems for environmental studies in future.

Author statement

Gulsah Congur: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization.

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Declaration of competing interest

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Appendix A. Supplementary data

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