Investigating gold nanorod-mediated hydrolysis of acetylthiocholine: A way for electrochemical detection of organophosphate pesticides

Chumki Praharaj[1], Smriti Singh[1,2], Pranav Tripathi[3], Seema Nara[1]*

*[1]Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, U.P., India

[2]Department of Biotechnology and Chemical Engineering, School of Civil, Biotechnology, and Chemical Engineering, Manipal University Jaipur, Jaipur, Rajasthan, India

[3]Department of Agriculture, School of Agriculture & Development, Central University of South Bihar, Bihar, India

*Corresponding Address: Dr. Seema Nara, Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Pryagraj-211004, U.P. India.

Ph.No. 91-0532-2271238.

E-mail Id: seemanara@mnnit.ac.in, seemanara@gmail.com

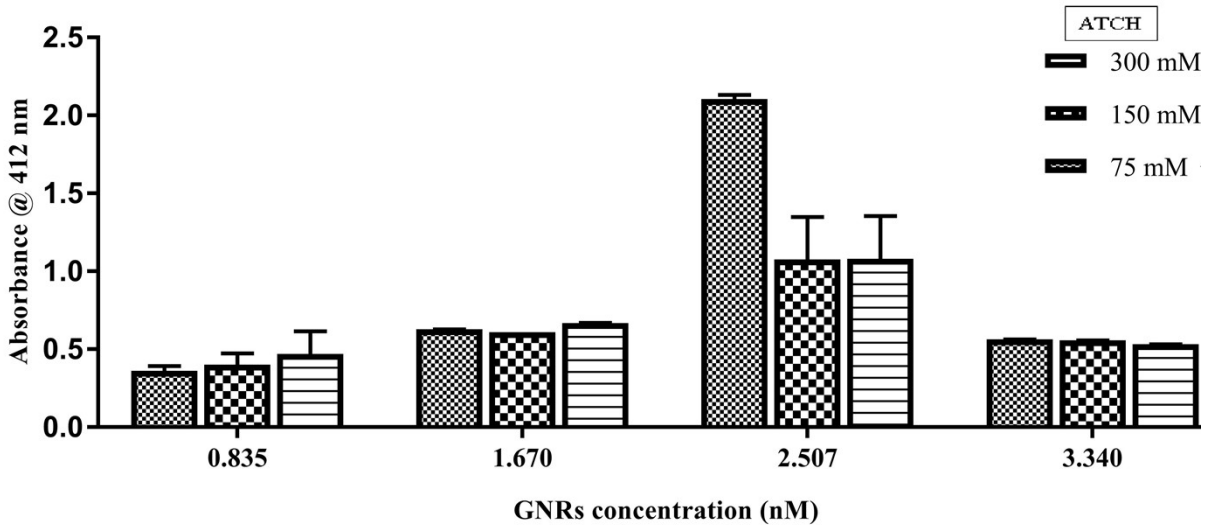


Figure S1. Optimization of GNRs and ATCH substrate concentration in GNR-based modified Ellman's assay.

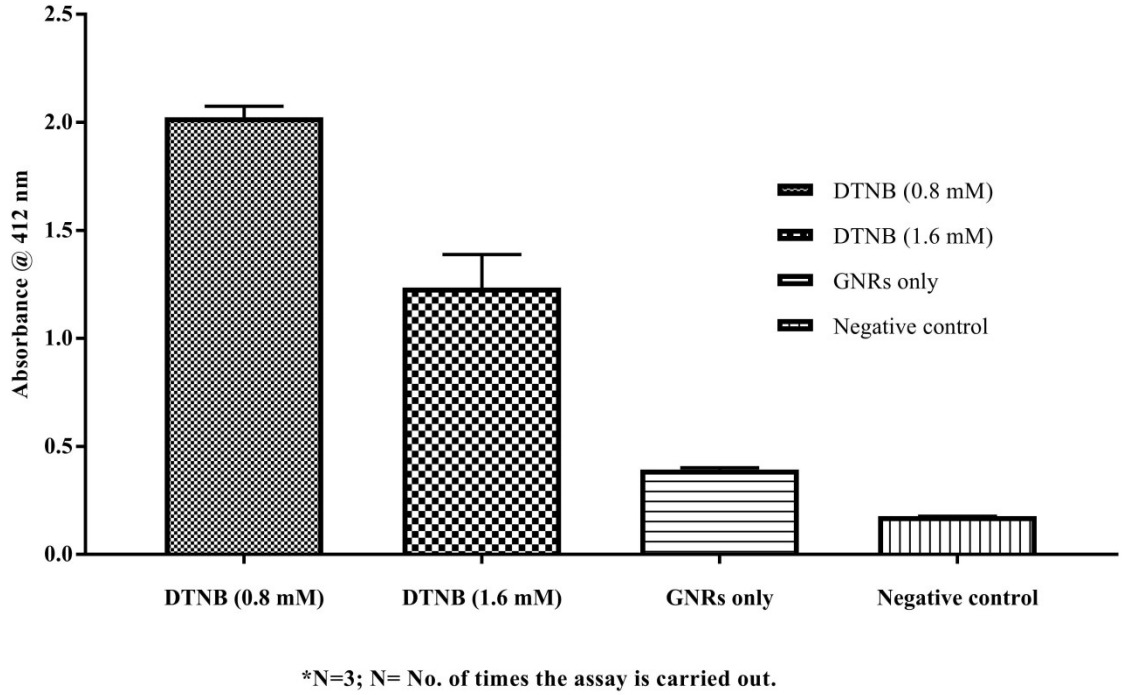
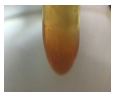
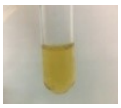
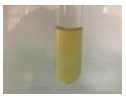


Figure S2. Optimization of DTNB concentration in GNR-based modified Ellman's assay.

Table S1. $FeCl_3$ mediated detection of acetic acid in various reaction permutations.

Enzyme used	Substrate	Formation of brown precipitate	Image	Inference
AChE	ACTH	✓		Breakdown of substrate to form acetic acid
GNRs	ACTH	✓		
AChE	ACH	✓		
GNRs	ACH	✓		
No Enzyme	ACTH	×		No conversion of substrate, no acetic acid forms
No Enzyme	ACH	×		-do-

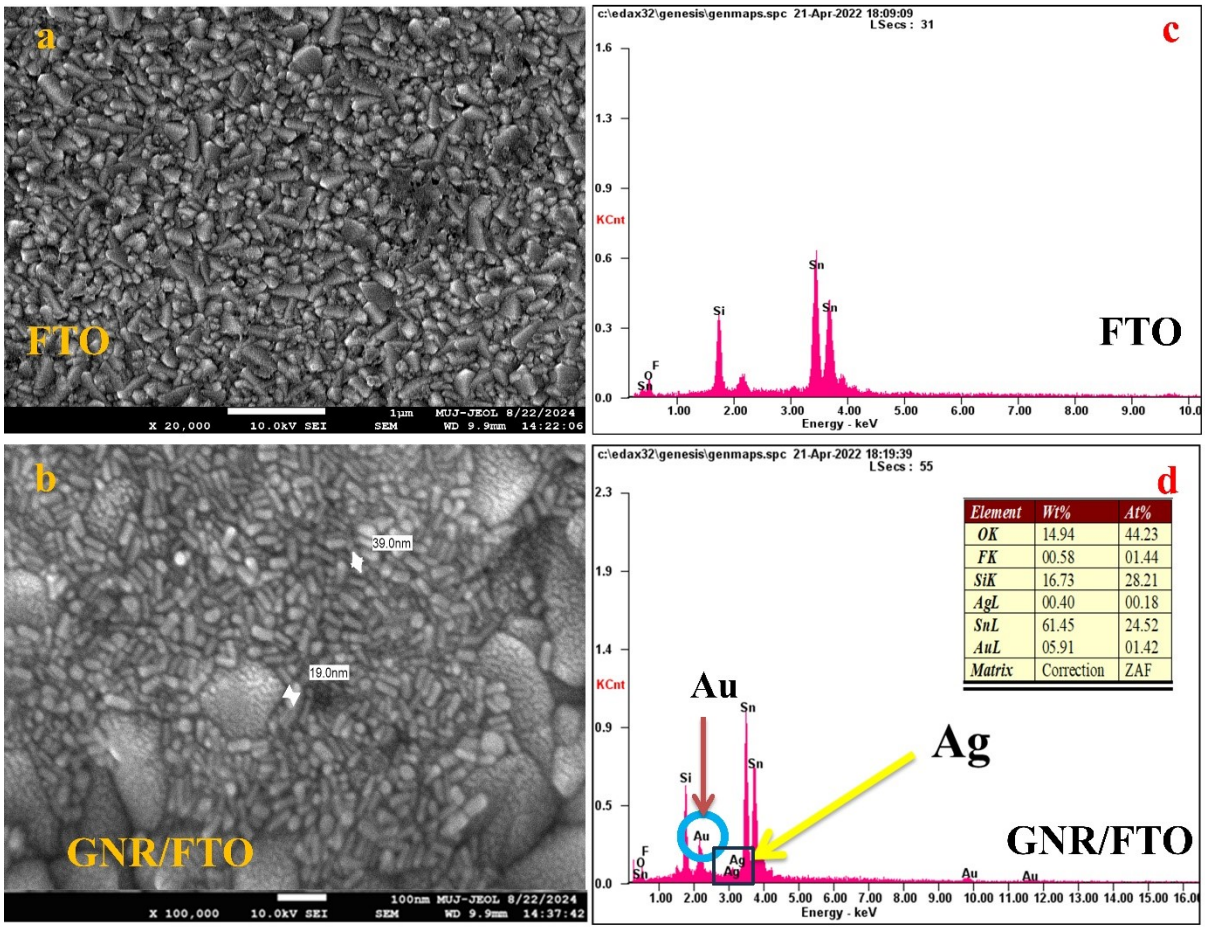


Figure S3. SEM images (a-b), SEM-EDX (c-d) graphs of Pristine FTO and GNR/FTO working electrode, and elemental distribution of GNR/FTO (in inset of figure S3d).

Table S2. Electrochemical parameters of Working electrodes (Only FTO (bare) and GNR/FTO) from cyclic voltammogram at different scan rates in 1×10^{-3} M Ferriferrocyanide with 0.5 M KCl as electrolyte.

Scan rate (mV/s)	**Oxidation Potential Epa (V)**	**Reduction Potential Epc (V)**	**Ipa (V)**	**Ipc (V)**	**Ipa/Ipc**	**ΔEp= (Epa-Epc)**	**Working Electrode**
10	0.3413	0.1764	0.3877	-0.3126	1.240	0.1649	Pristine
	0.3508	0.147	0.6064	-0.6002	1.01	0.2038	GNR
30	0.3738	0.1418	0.6375	-0.5027	1.268	0.232	Pristine
	0.3508	0.147	0.8490	-0.8404	1.01	0.2038	GNR
50	0.3874	0.129	0.7923	-0.6244	1.2688	0.2584	Pristine
	0.3508	0.147	1.2128	-1.2005	1.01	0.2038	GNR
70	0.4008	0.1111	0.9119	-0.7177	1.2705	0.2897	Pristine
	0.3508	0.147	1.3341	-1.3206	1.01	0.2038	GNR
100	0.4104	0.1013	1.0641	-0.8311	1.28	0.3091	Pristine
	0.3508	0.147	1.5161	-1.5007	1.01	0.2038	GNR
130	0.4221	0.0938	1.1887	-0.9257	1.284	0.3283	Pristine
	0.3508	0.147	1.6374	-1.6207	1.008	0.2038	GNR
150	0.4225	0.0853	1.2612	-0.9834	1.284	0.3372	Pristine
	0.3508	0.147	1.6980	-1.6807	1.01	0.2038	GNR

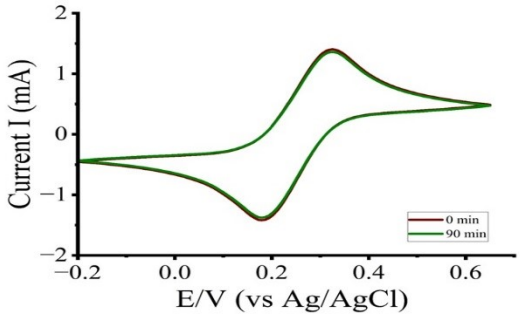


Figure S4. CV of GNR/FTO working electrode at 0 min (dark red line) and 90 min (green line) with 1.0×10^{-3} M Ferri-ferrocyanide and 0.5 M KCl electrolyte. (N=3. Where N* number of CV cycles).

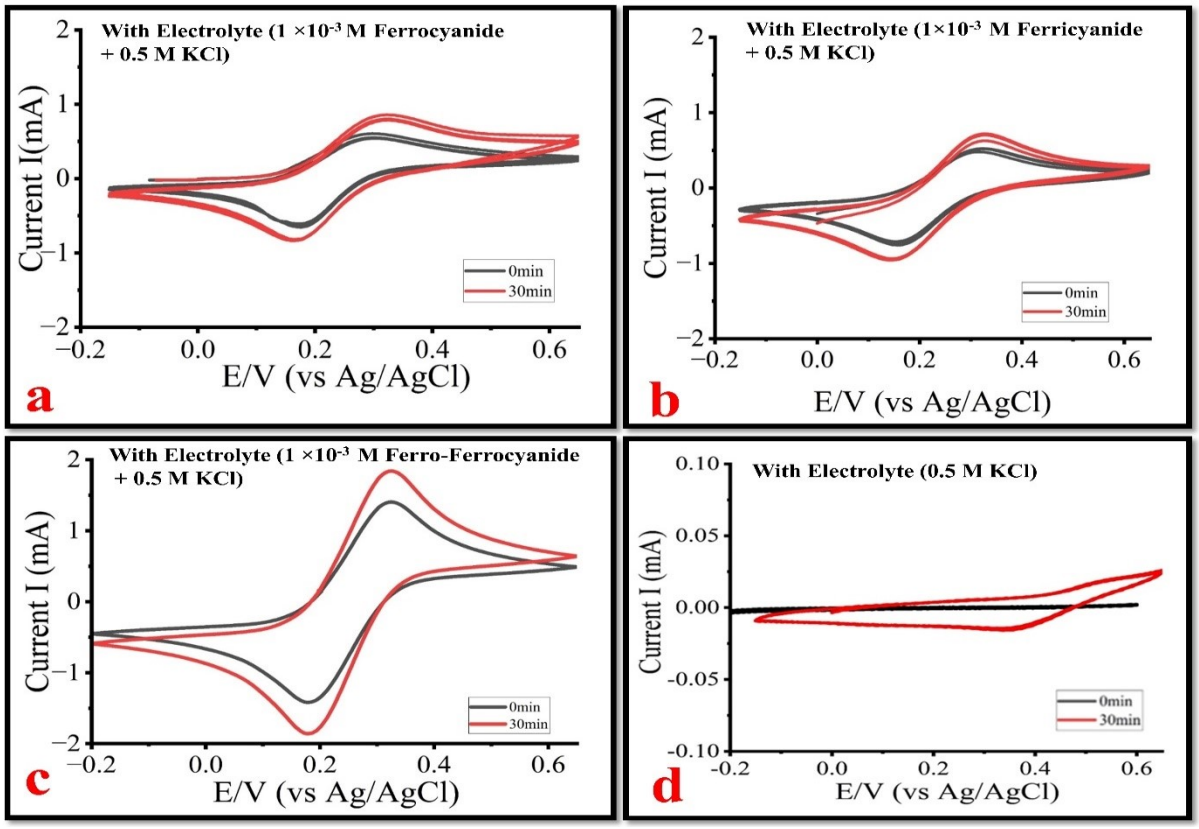


Figure S5. CVs of GNR/FTO with ATCH (substrate) in electrolyte having a) ferrocyanide + KCl, b) ferricyanide + KCl, c) both Ferri-Ferrocyanide + KCl, and d) KCl, at 0 min and after 30 min reaction time. (N=3. Where N* number of CV cycles).

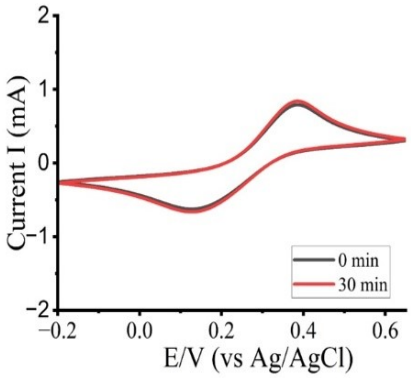


Figure S6. CVs of FTO with ATCH (substrate) in electrolyte having Ferri-Ferrocyanide at 0 min and after 30 min reaction time. (N=3. Where N* number of CV cycles).

Table S3. Interaction energy with two Au at LANL2DZ using effective core potential basis sets (ECP) for gold atom and B3LYP/6-31+G* for the rest of the atoms. The calculated interaction energy values are given in eV.

Molecule	**$E_{Molecule}$ (eV)**	**$E_{Complex}$ (eV)**	**E_{Au} (eV)**	**ΔE (eV)**
ACTH	-21886.43	-28118.34	-7312.355	-13544.271
ACH	-13098.52	-20461.42	-7312.355	-14675.254

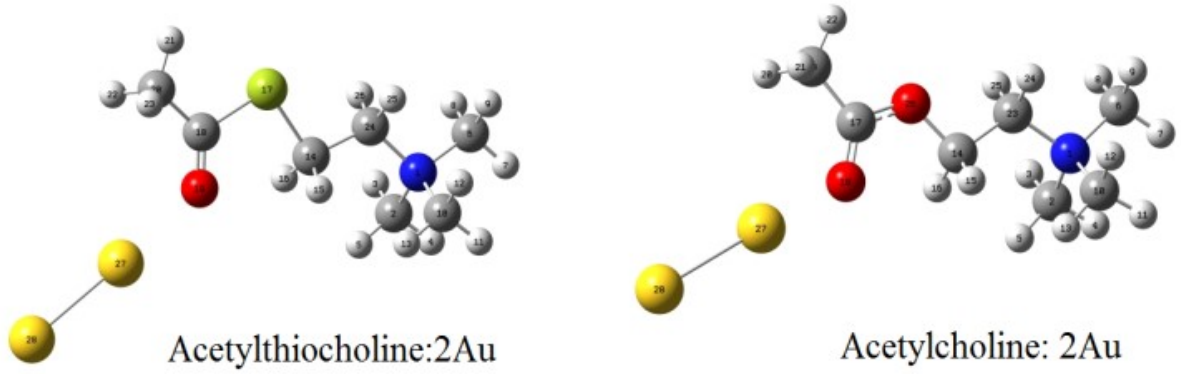


Figure S7. Interaction geometry with Acetylthiocholine:2Au and Acetylcholine:2Au with gold at LANL2DZ using effective core potential basis sets (ECP) for gold atom and B3LYP/6-31+G* for the rest of the atoms.

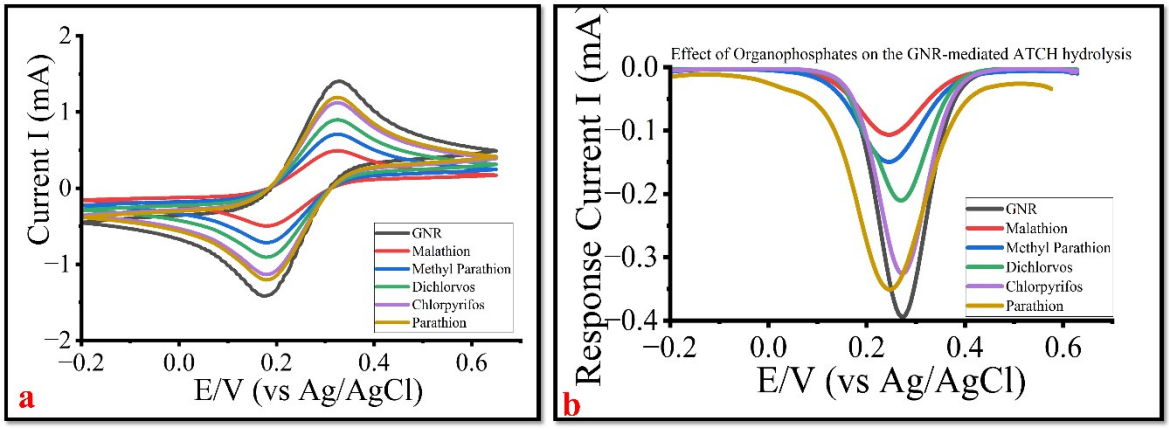


Figure S8. a) CV, and b) DPV of GNR mediated ATCH hydrolysis in the presence of various OPs (after 40 min of prior incubation of OPs with GNRs) (N=3. Where N* number of CV cycles and DPV).

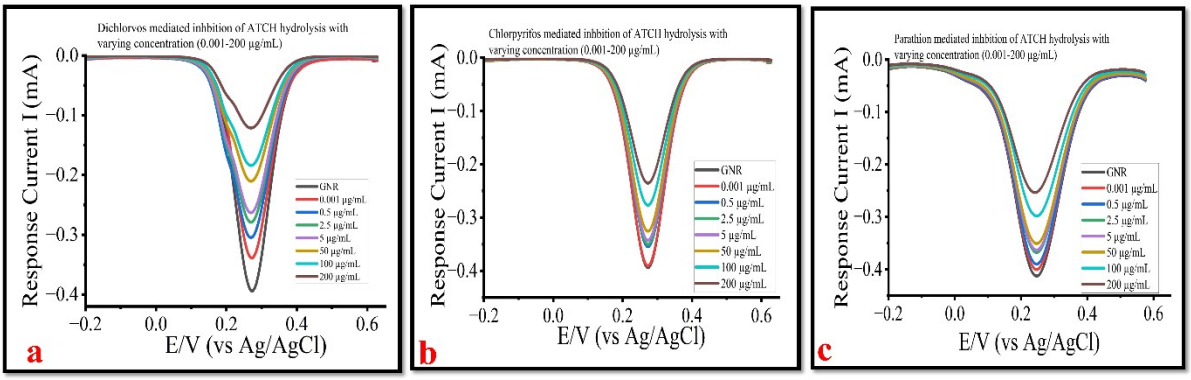


Figure S9. DPV response on varying concentrations of a) Dichlorvos, b) Chlorpyrifos, and c) Parathion. (N=3. Where N* number of CV cycles).

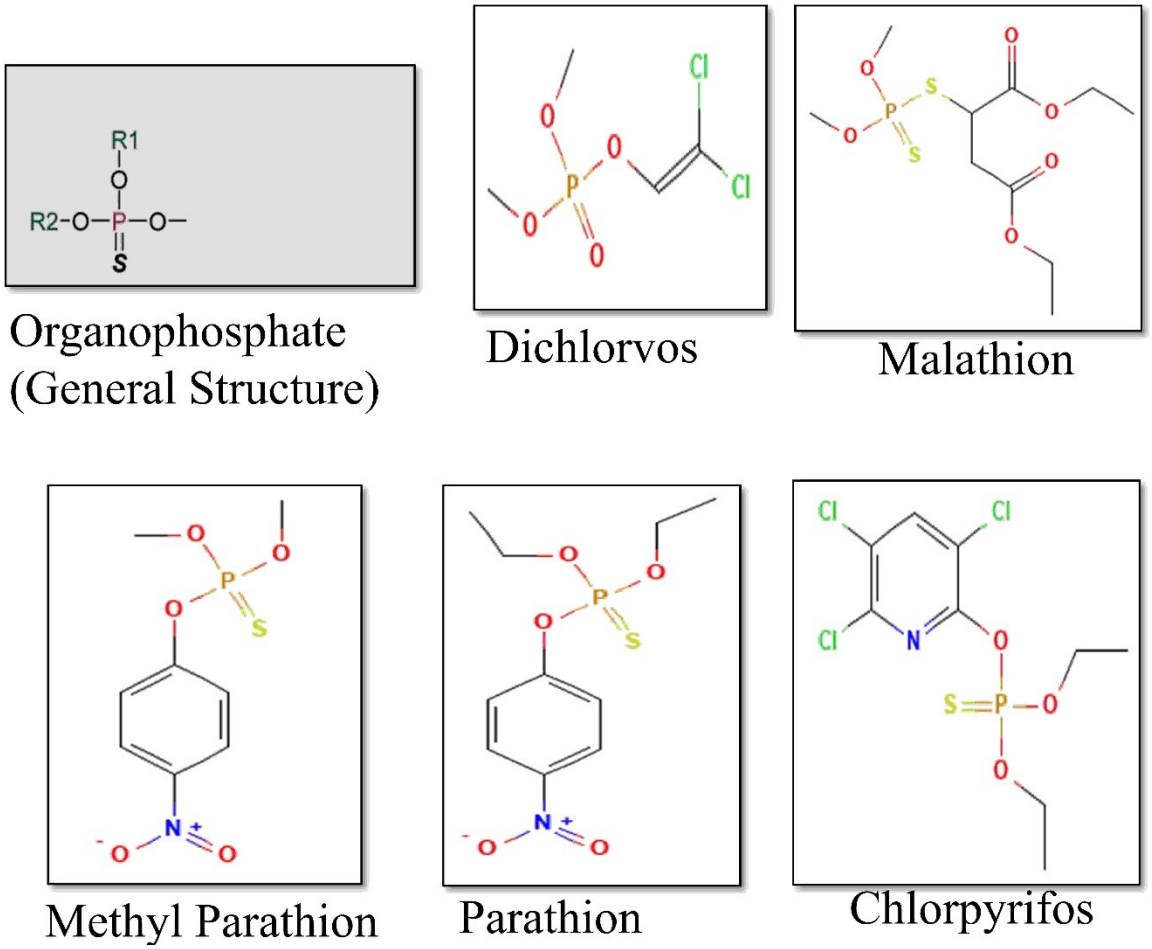


Figure S10. Basic chemical structures of Organophosphate (where R 1,2,3… are any alkyl group)[1], and representative of different class of organophosphate i.e. Dichlorvos (Orthophosphate)[2], Malathion (Dithiophosphate)[3], Methyl Parathion[4], Parathion[5], and Chlorpyrifos (Phosphorothionate)[6]. (Ref. http://npic.orst.edu/mcapro/opbiomarkers.html accessed on 14/02/2024)

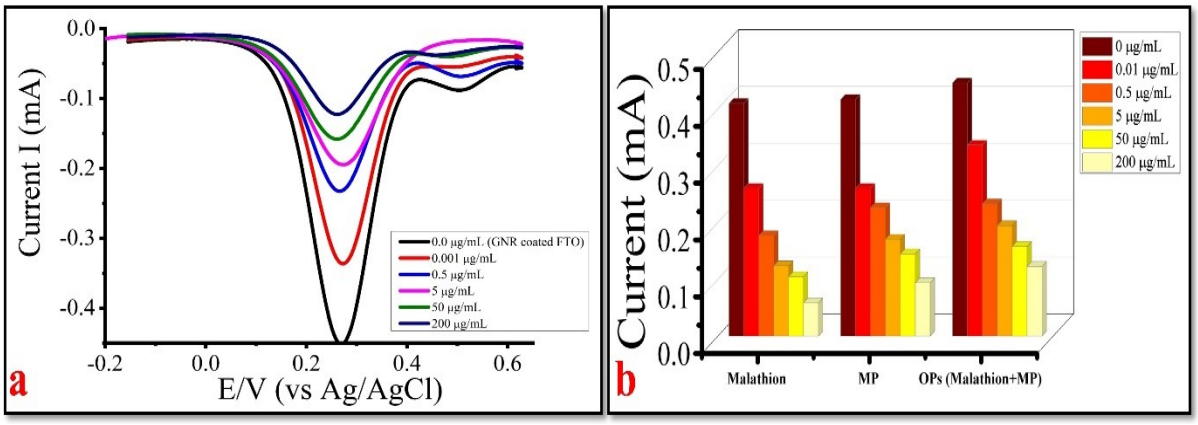


Figure S11. a) DPV of organophosphates (Malathion+Methyl Parathion) coated GNR/FTO, b) Comparison of OPs individual and mixed effect on GNR-mediated ATCH hydrolysis (OPs-organophosphates, MP-methyl parathion) (N=3. Where N* number of DPV cycles).

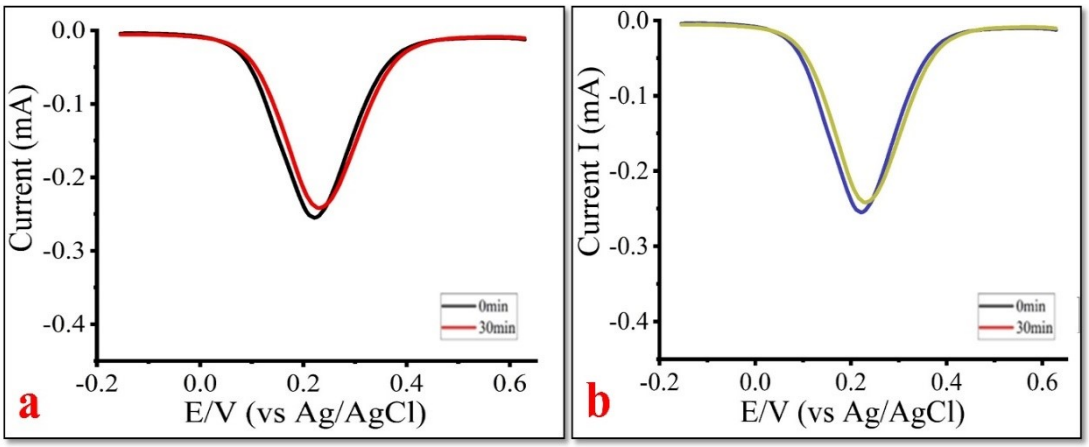


Figure S12. DPV of organophosphate coated FTO in the absence of ATCH a) GNR-Malathion/FTO, and b) GNR-Methyl Parathion/FTO (N=3. Where N* number of DPV cycles); Concentration of OPs used is 50.0 µg mL^{-1}.

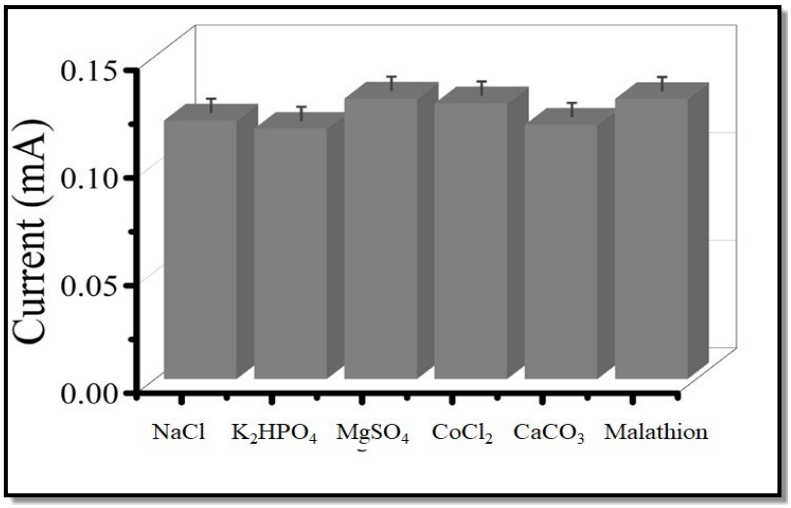


Figure S13. Selectivity of fabricated FTOs with common salts present in water resources. Error bars indicate the standard deviation for the three consecutive scans for DPV with R=0.9 for fabricated FTOs. (N=2. Where N is number of times assay was performed); with 50.0 µM concentration of interfering salts (interference).

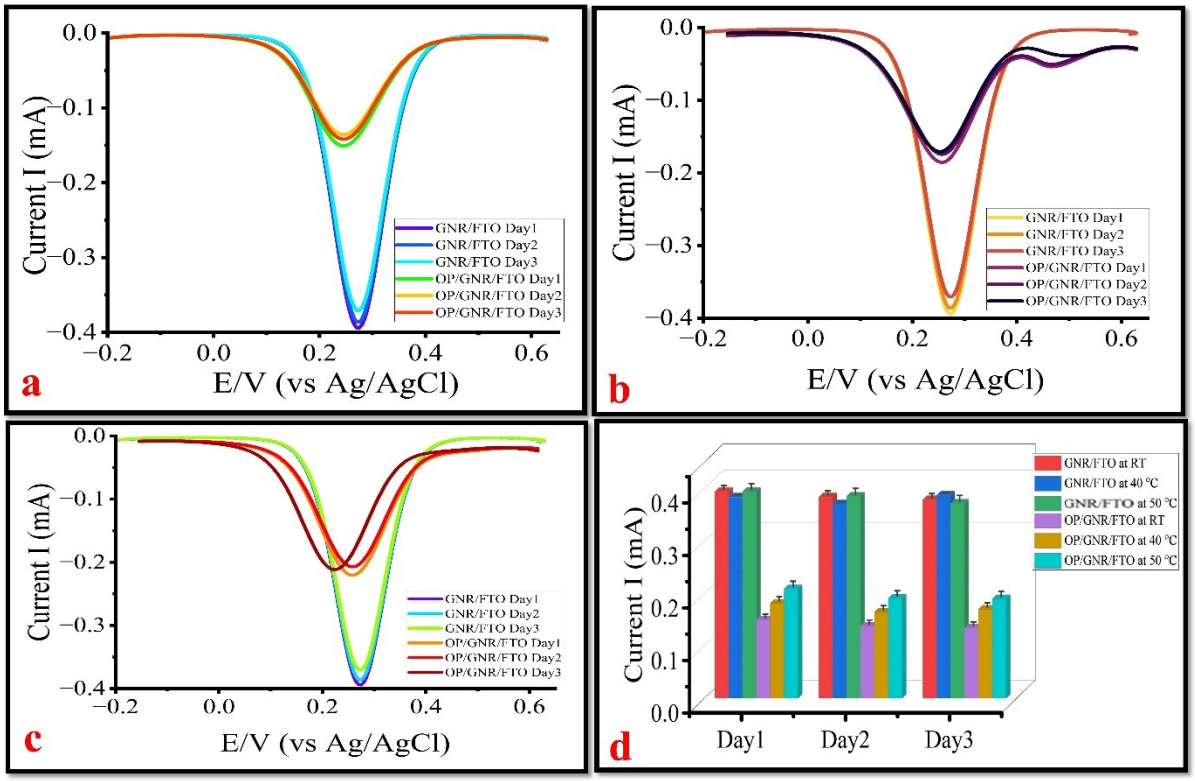


Figure S14. Stability of the GNR/FTO and GNR-OP/FTO. a, b, and c are DPV of fabricated FTOs at RT, 40 °C and 50 °C respectively, up to three days, and d is a bar graph of response

current vs time (Days). Error bars indicate the standard deviation for the three consecutive scans for DPV with R=0.9 for both WEs.

Table S4. Recovery data of spiked river (Ganga) water samples with OP (malathion)

Spiked Pool	**Spiked concentration ($\mu g\ mL^{-1}$)**	**Expected Concentration (E) ($\mu g\ mL^{-1}$)**	**Observed Concentration (O) ($\mu g\ mL^{-1}$)**	**% Recovery = (O/E) *100**
River Water (Non-spiked)	-	-	0.001	-
Low Spiked sample	0.001	0.002	0.002	100.0
Medium Spiked sample	2.5	2.5001	2.6	104
High Spiked sample	50.0	50.001	50.120	101.238

Reference:

1. http://npic.orst.edu/mcapro/opbiomarkers.html accessed on 14/02/2024.
2. https://pubchem.ncbi.nlm.nih.gov/compound/Dichlorvos accessed on 14/02/2024.
3. https://pubchem.ncbi.nlm.nih.gov/compound/Malathion accessed on 14/02/2024.
4. https://pubchem.ncbi.nlm.nih.gov/compound/Methyl-parathion#section=2D-Structure accessed on 14/02/2024.
5. https://pubchem.ncbi.nlm.nih.gov/compound/Parathion#section=2D-Structure accessed on 14/02/2024.
6. https://pubchem.ncbi.nlm.nih.gov/compound/Chlorpyrifos accessed on 14/02/2021.