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# Modern Methods of Measuring Pesticides Using Biosensors

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## Abstract

Because of the large amounts of commonly used pesticides as well as their impacts upon health and the environment, there is a high importance in finding an accurate and prompt pesticide analysis methods. This paper provides an overview for modern methods and trends in the measurement of pesticide contamination based on biosensors. Moreover, there are different types and classifications of biosensors which play a successful role in environmental and agricultural pollutant analysis, and process control. Present study, conducted on view the more development bio-receptors depending sensors in additional to fluorescent biosensor for pesticides detection. This review showed that biosensors depend on biological component (such as an enzyme, a DNA probe, antibody) as recognition elements/bio-receptors are more accurate and reliable than traditional analytical instruments. In conclusion, The application of the bio-sensor-based instrument to pesticide detections in environment and different biological products like the vegetables and fruits are successful and will be researched more in the future.

**Keywords:**

Pesticides, Biosensors, Agricultural, Environment, Analyte, Organophosphate, Transducer

[2,3]. The UN had estimated that 200000 death cases from the acute poisoning happen every year as a result of the use of pesticides and 99% of these cases belong to developing countries. Researches have shown that most pesticides are considered carcinogens, in addition to their effect on the immune system, bone marrow, liver, nervous, respiratory system, circulatory system, etc. Many health organizations have warned of the dangers of using these pesticides widely [4] .On the other hand, At the present time, the use of pesticides has increased with the increase in agricultural fields and animal husbandry, in addition to their use in homes to get rid of mosquitoes or flies [1]. Over time, insecticides have developed to be of a wide spectrum, as they have become fatal to insects, worms, and even bacteria and fungi. Where it has a negative impact on human, animal and plant life, through its entry into the food chain or through its direct entry into the human body

potentials for the future enhancements have been mentioned.

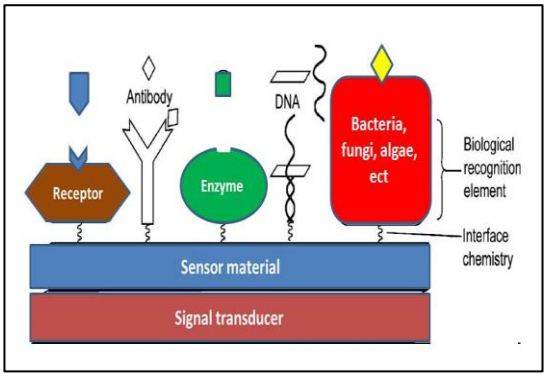



**Figure (1):** Components and method of biosensor response (reference; this study)

## Principle of Modern Methods that Based on Biosensor Instruments for Pesticide Detection

Principle of pesticide evaluation modern methods that represented by biosensor instruments depends on the combination of transducing device with an element of recognition, as shown in figure (1). Those instruments had something in common, like support material that elements of recognition have been immobilized on [12]. This element of recognition could be an anti-body/anti-gen pair, an enzyme as well as its substrate, a receptor with the specific ligand, or even analyte with the living cells which bind to them in particular. Specific interactions between target analyte and the biological-recognition element could result in producing physio-chemical alterations, which have been detected and measured by transducer [13]. Bio-chemical signal would be processed to digital electronic or analog signal via the transducer. Analyte concentration might be obtained from proportional correlation between the concentration of the analyte and the strength of the signal. According to this concept, quantitative detection of the residues of the pesticide may be obtained (Fig. 2). Throughout the past 10 years, more instruments of detection that are based upon the bio-sensors have been discussed in literature, like instruments of the pesticide residue detections [12, 14, 15]. The majority of the bio-sensor instruments that have been reported up until now utilize different concept of detection. According to the mechanism of many studies have tried for nearly a decade to find solutions to reduce the problem of pollution resulting from the utilization of the pesticides through the use of non-toxic materials that are not stored in human, animal or plant tissues, in addition to following methods for identifying and analyzing those pesticides [5]. Detecting pesticides at the levels that have been set by Environmental Protection Agency (EPA) is still challenging. The chromatographic approaches, like the High Performance Liquid Chromatography (HPLC), Gas Chromatography coupled with the Mass Spectrometry (GC-MS/MS), HPLC-MS/MS or combined with selective identification tools have long been utilized for analyzing pesticides as a result of their sensitivity, accuracy, reliability and efficiency [6]. However, it has disadvantages, the most important of which are laborious and time-consuming, also, they require expensive materials and equipment and highly trained specialists. Throughout the last 10 years, great attention was given to developing bio-sensors to detect insecticides as a promising alternative [7].

Recently, numerous researches have focused on the use of new technologies in this field, most notably the biological sensor. A bio-sensor is a self-contained device which integrates a moving biological component (like an enzyme, an antibody or a DNA probe) which recognizes analyte (eg enzyme substrate, anti-gen, or complementary DNA) and the transducer that has been deployed for the conversion of (bio) chemical signal that is generated by analyte's interaction with bio-receptor into electronic device [8,9]. Based on the technology of signal transmission, the bio-sensors are classified to electro-chemical, piezoelectric, optical and mechanical bio-sensors. The electro-chemical transformers were commonly utilized in the bio-sensors in order to detect the pesticides, because of its high sensitivity [10]. In addition, its simple design, low cost, and small size have made them very good candidates to develop portable bio-sensors [11]. Depending on biological recognition element, enzymatic, whole cell, immunohistochemical and DNA bio-sensors are designed for the detections of pesticides as in fig. (1). In this review article, we summarize and discourse modern biosensors that are best in measuring pesticide levels or concentrations, which in turn are important for environmental, agricultural and food control. Furthermore, aspects that are associated with analytical performance of developed biosensor together with the

sensor and mass based bio,sensor according to the mechanism of the transduction. Optical Biosensor involved two branches are magnetoeistic and piezoelectric sensors the latter contains surface acoustic waves-based (SAW) and a quartz crystal microbalance immunosensor (QCM) [12,20,21]. Electro-chemical bio-sensors may as well be categorized into potentiometric, amperometric, impedimetric and conductometric bio-sensors, based upon characteristics of output signal that has been obtained from the transducers (Figure 3). In addition, the optical biosensor includes a set of devices, which include: Surface plasmon resonance (SPR), Fiber optic, Raman and Fourier transform infrared (FT-IR) [21,22]. The instrument of the pesticide residues detection based upon the bio-sensors has been one of currently hot areas of the research that have complied with sensitive and fast detection technology requirements [22].

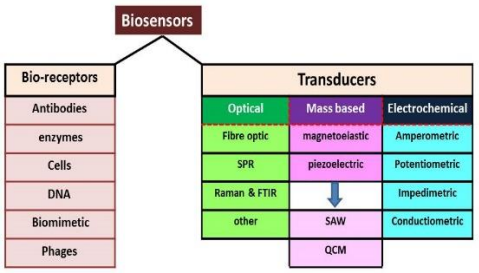
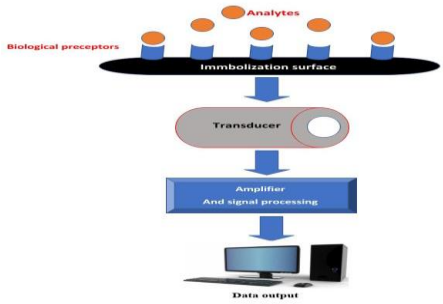



Figure3: Classification of Biosensors (reference; this study)

## Advanced and Widely Used Biosensors Today for Pesticides Detection

After the insistence of the World Health Organization on the need to implement the goals of sustainable development, the most important of which is the control of pollutants, and as we mentioned, one of the most dangerous of these pollutants is pesticides. To achieve this requirement, most scientific research and applied studies have directed the development of modern methods to determine the percentage of pollutants in water, air or soil that are added to tissues. Among the most prominent of these methods are biological sensors that depend on enzymes, nucleic acids, microbes, antibodies, or nanoparticles for their work. Recently, the focus has become on biological sensors as they are available, accurate, easy to use and

the transduction, the bio-sensor-based tool might be classified additionally to optical instrument, electro-




chemical instrument and mass-based bio-sensor instrument, which will be described in the upcoming sections [16].

Figure (2): The Basic installation of the biosensor device (reference ; this study)

## Recent Classification of the Biosensors

The bio-sensors represent a part of latest classification of the sensors that have been presented by International Union of Pure and Applied Chemistry (IUPAC) [17,18]. Bio-sensors applied biological elements to be integrated electronic device and those biological elements may be nucleic acids, receptor proteins, anti-bodies, cells, enzymes and tissue sections as medium of analysis. For the purpose of realizing the detection of the signal, an element of the biological recognition has been combined with the bio-sensor for the detection of the signal to transducer, (Figure 3) that may finally be shown on biosensor-based instruments panel after the processing and amplification of a signal [12,18]. Combining biological molecules with the electronics had a significant impact on further bio-sensor development. Based on biological-recognition element or transducing system that are utilized, bio-sensors which may be categorized to various types [19]. Based upon various biological-recognition element types, the bio-sensors may be categorized to enzyme-based bio-sensor, cell-based bio-sensor, immunosensor, bio-mimetic bio-sensor, microbial biosensor, phage-based biosensor, etc. [12,20]. The selectivity and specificity of the bio-sensor have been based upon biological-recognition element that has been highly impacted by transducer. The bio-sensors may be classified additionally to optical bio-sensor, electro-chemical bio-

procedures have been utilized as well in the bio-sensors. The immobilization of an enzyme on transducers is one of the crucial steps in bio-sensor development. The simplest immobilization form is enzyme dissolution in buffer solution, its deposition on surface of the electrode and covering it by dialysis membrane [26]. Other methods of immobilization have been based upon enzyme's physical entrapment, within synthetic gel layer (which is formed by copolymerization of the bisacrylamide and acrylamide) or chemical bond between enzyme and membrane or inorganic or organic support or directly to transducer (which is made up of Au, C, Pt, and so on). This enzyme may as well be immobilized through the cross-linking with inert protein with the gluter-aldehyde and forming the insoluble macro-molecular aggregates [26,27]. Numerous bio-sensors (i.e. enzyme-based ones), utilized for the detection of the pesticides are catalytic activity based or are inhibition of reaction, of many enzymes in presence of the pesticides [25].

The properties of the inhibitions and re-activation of bio-sensor by organo-phosphate pesticides have been researched [28]. The re-activation of immobilized enzyme reactor has been reported for determining acetyl-cholinesterase inhibitors in the mode of the flow injection utilizing 2-pyridinealdoxime Methochloride. Pralidoxime iodide has been utilized as well, as an agent of reactivation for inhibited AChE enzyme [29,30]. Table1 and Table2 summarize the most widespread enzymatic bio-sensors for pesticide detection.

can be developed. We have noticed the success of many studies in discovering many enzymes, microbes and nanoparticles that can be used as a receiver or sensor for toxic chemical elements in insecticides or pests. Given the interest of the current study in determining the latest methods for determining the percentage of pesticide contamination, we will discuss the sensors that have been successfully used in the field of work from hand, and follow up on new experiments to improve their efficiency and facilitate their use on the other hand.

## Enzyme-Based Biosensor for Pesticides Detection

The enzymes can be defined as organic catalysts that are produced by living cell acting on substances that are referred to as the substrates. Similar to all of the other catalysts, the enzymes only catalyze the reactions that are thermo-dynamically possible [23]. Enzyme-based bio-sensors evaluate the Enzyme-catalyzed reaction rate as a base for their responses, any of the physical measurements that produces an amount which is related to that rate may be utilized for the detection. Numerous procedures were devised in order to monitor enzyme activity with the use of the electro-chemical transducers [24, 25]. The evaluation of that activity typically occurs by directly measuring electro-active products or co-substrates that are involved in enzymatic reactions [25]. There is a possibility for realizing this monitoring in an indirect way as well, with the use of the synthetic mediators which favor electron transfer between electro-active species and the electrode. Those

**Table1.** Enzymatic bio-sensors for the direct pesticide detection [25]

| ANALYTES | ENZYMES | LIMIT OF DETECTION | SYSTEM |
|---|---|---|---|
| Di-isopropyl Fluorophosphates | OPAA | 20μM and 12.5μM for the batch and flow injections | Amperometry |
| Parathion / Paroxon | Parathion hydrolase | 15nM/ 20nM | " |
| Parathion | Parathion hydrolase | 1ng/ml | " |
| organophosphorus neurotoxin | OPH | 2μM and 6μ for the paraoxon dichlorvos, respectively (i.e. potentiometry) and 70 nM for paraoxon (i.e. amperometry) | Amperometry/potentiometry |
| Paraoxon / methyl parathion | OPH | 20 nM | " |
| OPAA, organo-phosphorus acid anhydrolase; OP, organo-phosphorus; OPH, organo-phosphorus hydrolase | | | |

Table 2. Enzymatic bio-sensors for the indirect pesticide detection [25]

| ANALYTES | SUBSTRATES | ENZYMES | LIMITS of DETECTION |
|---|---|---|---|
| Tri-chlorfon | BuCh | BuChE | < 0.1 µM |
| Coumaphose/tri-chlorofon/ aldicarb/methiocarb. | TCh | TChE | 0.002/ 0.40/ 0.30 / 0.08µg/ml |
| Carbaryl | ATCh | ATChE | 5.0"10$^{-5}$mol/l |
| Carbafuran | ATCh | ATChE | at nM |
| Malthion | " | ATChE | 0.03ng/ml |
| Triazophose | " | ATChE | 0.01 µM |
| Paraoxon / chlorpyrifos ethyloxone | P-aminophenol | ChE | 19.1nM/ 1.24nM |
| chloropyrifosmethyl/ coumaphos/ carbofuran | TCh | TChE | 2x10$^{-8}$, 5x10$^{-8}$ and 8x 10$^{-8}$M |
| Dichlorvos/ Carbofuran | ACh/Ch | ChE/ChO | 1.30 x 10$^{-3}$ /0.01ppb |
| Carbofuran / paraoxon | ACh / BuCh | AChE/BuChE | 0.2nM and 0.60nM |
| Chlorpyrifos ethyl oxon | TCh | ChE | 1 ppb |
| Aldicarb | Glucose-6-phosphate/ glucose | AP/GOD | 40µg/ml |
| Carbaryl / Carbofuran | " | AChE/ChO | 2µg/ml |
| Trichlorofon | ACh/Ch/ $H_2O_2$ | ChE/ChO/peroxidaze | 5ng/ml |
| Triazine | " | Tyrosinase | 0.5x10$^{-9}$mol/l |
| Methyl parathion/diazinon/Carbofuran/Carboy | Monophenol | Tyrosinase | 6ppb/19ppb/5ppb/10ppb |
| Zineb | Propinoaldehyde | Aldehyde dehydroganse | 8ppb |
| Diazinon /dichlorvos | " | Tyrosinase | 5µM and 75µM |
| Ziram/ diram/ zinc diethydithiocarbamate | " | Tyrosinase | 0.0740/1.30/1.7µM |
| Ethyl paraoxon | Ascorbate | Ascorbate oxidase | 1ppm |
| Herbicide | Pyruvate | Acetolactae synthase | 1µM |

ACh, acetylcholine; BuCh, butyrylcholine; ATCh, acetylthiocholine; BuTCh, butyrylthiocholine; Ch, choline; TCh, thiocholine; BuTChE, butyryl thiocholine estrase; ATChE, Acetyl thiocholine esterase; ChE, choline estrase; ChO, choline oxidaze; GOD, glucose oxidaze; AP, acid phosphates.

A sensitive electro-chemical bio-sensor for the carbofuran (CBF), based upon acetyl-cholinesterase (AChE), with modified vitreous carbon electrode, a multi-walled carbon nanotubes (MWCNT) and polyaniline shell (PANI), has been assembled by Martinez et al. GC/MWCNT/PANI/AChE Bio-sensor had shown 1.4mol/L detection limits for the CBF and has been successfully applied in the samples of Cabbage, apples and broccoli. Results have been validated via the HPLC [33]. Concerning amperometric bio-sensors, Montes etal. Have described graphite-epoxy-AChE bio-sensors' characterization and optimization, improving required electro-chemical characteristics, like the High rate of electron transfer, a high signal-to-noise ratio, and sufficient sensitivity. Analyzed Samples of the water have shown a 0.00025mg/L LOD for CCB. The insecticide chlorpyrifos (ChP) has been analyzed with

## Biosensors Based upon the Acetylcholinesterase Inhibition via Various Pesticides

A bio-sensor based upon cholinesterase inhibition activity is quite commonly utilized in determining organo-phosphate pesticides, like the chlorpyrifos. The analytical device incorporating enzyme is integrated to physicochemical signalling transducer or transduction micro-system [31]. After that, the signal (i.e. optical detector, electrode, piezoelectric crystal, and so on) performs the conversion of bio-chemical response to amplified optical and electric signals, decoded and measured through suitable electronic unit. Through the use of this approach, the analyte performs the selective inhibition of immobilized enzyme activity, which leads to in the reduction in the signal that proportionate to target analyte amount which is present in solution [32].

organo-phosphorous bio-sensor [35]

## Cell-Based Biosensor for Pesticides Detection

Living micro-organisms (bacteria, algae, fungi and yeast) may be utilized as bio-catalytic elements for the bio-sensors [38]. Microbial (pieces of cells and whole cells) bio-sensors could be less expensive and simpler for developing for some of the applications, which eliminate the necessity for isolating and purifying enzymes as well as the related cofactors needed required for the enzyme- based bio-sensors [25].

The amperometric microbial bio-sensor for directly determining the p-nitrophenyl-substituted organo-phosphate has been developed. The bio-sensor has been comprised of the p-nitrophenol degrader, Pseudomonas putida JS-444, which had been genetically engineered for the expression of OPH upon cell surface that is immobilized on an electrode of carbon paste [39]. Electro-oxidization current of intermediates has been measured and associated with OP concentration. The limits of the detection have been similar to the cholinesterase inhibition-based bio-sensors. Under the optimal operation conditions the bio-sensor measured as low as 0.26ppb, 0.28ppb, and 0.29ppb of the methyl parathion, paraoxon, and parathion respectively. A conducto-metric bio-sensor utilizing the immobilised Chlorella vulgaris micro-algae as bio-receptors has been as bi-enzymatic bio-sensor. The algae has been immobilised within the membranes of the bovine serum albumin that have been reticulated with the glutaraldehyde vapours that was deposited upon the inter-digitated conducto-metric electrodes [40]. Local variations of the conductivity that result from the activities of the acetylcholinesterase and algae alkaline phosphatase may be detected. Those OP pesticides for the acetylcholinesterase, bi-enzymatic bio-sensors have been tested for the purpose of studying the impact of the pesticides and ions of heavy metals upon corresponding enzyme. For the pesticides, the initial experimentations have shown that the paraoxon-methyl inhibits the Chlorella vulgaris AChE unlike carbofuran and parathion-methyl [25].

An amperometric microbial bio-sensor for direct OP nerve agent measurements has been described. This sensor has been based upon carbon paste electrode that contains the genetically engineered cells that express the use of self-assembled monolayer (SAM) electrode of the single-walled carbon nanotubes (SWCNT), which has been wrapped by thiol-terminated single-stranded Oligonucleotide (ss-DNA) in gold, in a poly-aniline matrix for immobilizing the enzyme, Acetyl-cholinesterase. This bio-sensor has a vital step, where a small variation of the pH happens in vicinity [33,34].

## Principle of Organophosphorous Pesticides Bio-sensor Based upon AChE Inhibition Mechanism

Bio-sensor's sensitivity is dependent upon bio-recognition layer that catalyzes the reaction. The product/byproduct further or itself plays the role of a signal that is inversely or directly proportional to concentration of analyte. In a cases of the Ache inhibition based organo-phosphorous (OP) bio-sensors, the produced signal is inversely proportionate to OP compound concentration or, in other words, it can be said that the increase in the OP compound concentration produces weak signals. The bio-sensor of the AChE mainly works on the effects of the inhibition [35] which can be seen from figure (4). The bio-sensor where AChE has been utilized as an element of the bio-recognition has the ability of detecting toxic OPs along with others like the nerve agents, carbamate pesticides, and many other natural toxins [36]. Some of the medications may as well be detected using such bio-sensors [37]. In the case where the inhibitor isn't present in sample then acetyl-thiocholine will be converted to thiocholine and acetic-acid. However, in the case where inhibitor in sample then thiocholine concentration is reduced or no acetic-acid or thiocholine is produced, i.e. it inhibits the conversion entirely. Under an impact of the applied voltage thiocholine is oxidised. The anodic current of oxidation is inversely proportionate to toxic compound which is present in sample and exposure time [35].

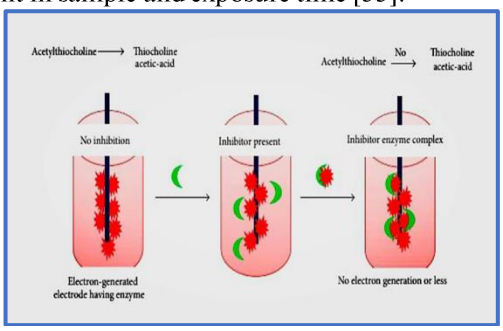



**Figure (4):** Principle of the AChE inhibition-based

different DNA compounds that have been immobilized onto the respective electrodes. A review article for the electro-chemical DNA bio-sensors has been published as well on that subject [46,47].

Double-strand calf thymus deoxyribonucleic acid entrapped polypyrrole-polyvinyl sulphonate (dsCT-DNA-PPy- PVS) films that have been fabricated onto the indium-tin-oxide (ITO) coated plates of glass has been utilized for the detection of the OPs like the malathion and chlorpyrifos [48]. Those bio-sensing electrodes have a 30s response time, they have stability for approximately five months in the case of being stored in the desiccated conditions at a temperature of 25°C and may be utilized for the amperometrical detection of the chlorpyrifos (0.0016ppm-0.0250ppm) and malathion (0.17ppm - 5ppm), respectively. the bio-sensors of DNA have been based upon the PANI-PVS and produced with the uses of the electrochemical entrapment method into the ITO for detecting the OP pesticides (i.e. malathion and chlorpyrifos) [49]. Those double stranded calf thymus bio-electrodes have been identified with the use of the square wave voltammetry, FT-IR spectroscopy, SEM and electro-chemical impedance methods, respectively. Those dsCT-DNA entrapped PANI- PVS/ITO bio-electrodes had been found to be having a 30s response time, with an approximate 6-month stability and detection limit 0.01 ppm and0.5ppb for the malathion and chlorpyrifos, respectively [25].

## Electrochemical Immunosensors for Pesticides Detection

The concept has been based upon electrical characterisrics of buffer or electrode affected by the interaction of Ab-Ag. They could lead to the determination of the pesticide level through the measurement of changes of the current, potential, impedance or conductance that result from immuno-reactions [50]. Anti-gen (i.e. pesticide- protein conjugate) is initially immobilized on transducer surface, and after that, the mixes of the pesticide-antibody are pre-incubated in the solution. Following the injection upon the surface of the sensor, the anti-body binding to immobilized conjugate undergoes inhibition by existence of target pesticide [50].

The impedimetric immuno-sensor has been developed in order to determine the atrazine [51]. This the OPH on the surface of a cell. The organophosphorus hydrolase catalyzes OP pesticide hydrolysis with the substituent of the p-nitrophenyl like the parathion, paraoxon, and parathion-methyl to p-nitrophenol. The latter can be anodically detected at carbon transducer with the current of oxidation being proportionate to concentration of nerve-agent. Microbial bio-sensor had exceptional stability of the storage, which retains 100 % of the original activity in the case of being stored at a temperature of 4°C for a 45-day period [41].

The bio-sensor has been constructed through the deposition of cultured E. coli cell suspension onto poly-carbonate membrane and mounting this membrane upon glass electrode through O-ring [42]. The bio-sensor response for parathion, paraoxon, diazinon and methyl parathion has been studied. The impacts on the response of the concentration of the buffer, temperature and pH have been reported. The graphs of the calibration have not been linear and the limits of the detection for all analytes have been 3μM [42].

Amicrobial bio-sensor which includes the dissolved oxygen electrode that has been modified with genetically engineered PNP- degrader Moraxella sp. That display organo-phosphorus hydrolase on cell surface for the selective, sensitive, direct and rapid determinations of p-nitrophenyl (PNP)-substituted or-ganophosphates (Ops) has been reported. Operating at the optimal conditions the bio-sensor has been capable of measure a minimum of 27.50ppb of the paraoxon and had very good selectivity from the carbamates, triazines, and OP without the substitutents of the PNP [43]

## DNA-Based Bio-sensors for Pesticides Detection

DNA bio-sensors that are based upon the oxidation of the guanine were suggested lately for pesticide detection [44]. Those DNA sensors use DNA molecule interaction with a variety of the compounds either through the monitoring of the changes in DNA redox characteristics (in other words, guanine oxidation) or with electroactive analyte that has been intercalated on a layer of the DNA [45]. The electro-chemical methods, like the voltammetry and potentiometry were utilized for the purpose of studying interaction of

voltammetry [52]. Electrical resistance, Rm gradually reduces after every one of the buildings step of sensing membrane. The previous study results have shown that the immuno-sensor that has been based upon this approach has sensitivity to the atrazine anti-gen, as well as a good linear response in a 10ng/ml – 300ng/ml range. Table3 lists a summary of the variety of the immuno-sensor types that have been utilized in pesticide detection [52].

approach has been described for electro-chemical immuno-sensor development, for analyzing the atrazine that is related to the biotinylated-Fab fragment K-47 anti-body. Sensors have been based upon the mixed self-assembled mono-layer that consists of 1, 2-di-palmitoyl- sn-glycero-3-phospho-ethanolamine-N-(biotinyl)(biotinyl-PE) and 16-mercapto-hexadecanoic acid. The mixed mono-layer characteristics have been identified with the impedance spectroscopy and

Table3. Immunosensor Detection of the Pesticides [25]

| ANALYTES | IMMUNOSENSORS | SYSTEMS | DETECTION LIMITS |
|---|---|---|---|
| Simazine | Peroxidase label anti-body | Potentiometry | 3ng/ml |
| Paraoxon | Paroxon antibodies | Amperometry | 12ng/ml |
| Chlorsulfuran | Anti-chlorsulfuron antibodies | " | 0.01ng/ml |
| 2,4D | 2,4-D | " | 10PM |
| 2,4D / 2,4,5T | monocolonal/ polyclonal antibodies | " | 5 / 10PM |
| Paraoxon / methyl parathion/parathion | (Cell-based bio-sensor) microbial(Pseudomonasputida JS444) | Amperometry | 0.280/ 0.260 /0.29ppb |
| Atrazine | Biotinylated-fabfragement K47 antibody | conductommetry | 10ng/ml |
| Chlorpyrifos /malathion | (DNA) (Calf thymus-DNA) | Amperometry | 0.00160 / 0.17ppm |
| Paraoxon /parathion, methylparathion /diazinon | cultured of cells of E. coli | Potentiometry | 3.0µM |
| Paraoxon | Amicrobial (PNP- degrader Moraxella) | Amperometry | 27.5ppb |
| Chlorpyrifos / malathion | Double stranded calf thymus-DNA | Voltammetry, FTIR, SEM, and electrochemical impedance | 0.5ppb and 0.01 ppm |

## Fluorescent Biosensor to enhance the bio-sensor performance

As residues of the pesticide had resulted in some global concern about the environment and people's health, a precise and fast approach of detection has to be the maximum priority [53].. Carbon dots (CDs) that have been obtained from the cauliflower precursor and hydro-thermal synthesis approach have been synthesized as nano-sensor for the detection of the pesticides. The most promising benefits of the CDs-based fluorescent sensors are the fact that they're non-toxic, easy to form, time-saving, and cost-effective for the industrial applications. Synthesized dots of carbon have a possibility for considerably quenching the intensity of fluorescence emission in presence of pesticides [54]. The detection limit has been estimated to be 0.25ng/ml, 2ng/ml and0.5ng/ml for the diazinon, glyphosate and amicarbazone respectively, which has been considered as minimal detection limit amongst the typical approaches of the detection, like various types of the capillary electrophoresis, chromatography, and carbon dots detections in earlier researches. The prepared sensor's selectivity may be confirmed by absence of CD fluorescence quenching, in the case of their interference with the bromacil and dialen super. Synthesized CDs are capable of detecting the glyphosate, diazinon and amicarbazone [55]. In addition to that, the existence of 3 pesticides in one sample had as well exhibited quenching increase. None-the-less, there is a possibility that the CDs might not have the ability for distinguishing pesticides in a sample that contains the 3 pesticides. Moreover, this approach may be implemented in order to detect of those compounds in the fruits as well as other agricultural products. In addition to that, it's expected

that through world-wide utilization of such nanosensor, numerous infected consumable products may be found and the people's wellbeing would have gained promotion [56].

## Present and Future Work to Improve the Efficiency of Biosensors

There are many attempts to improve and develop the performance of the biosensor, which provides a better service in identifying pesticides. These attempts are represented in several directions, including improving the sensor, the distinct surface, or the electrical signals, in addition to linking the sensor to modern transmission networks that are connected to the Internet. Some scientific applications also dealt with controlling the size of the sensor and harnessing it to facilitate its use in various fields of work, and through this topic we will address the most prominent of those attempts below:

- Development of the miniaturized, multi-biosensors and utilization of the nano-materials keeps drawing many research efforts, which continue trends that have been indicated in earlier reviews that have been focused on the bio-sensors for the pesticides. The aim is reducing gap between the standard approaches, in addition to the acceleration of path toward the commercial implementations. None-the-less, the number of the applications that involve the actual environment or food samples and their varieties are still limited and the toxic metabolites of the pesticides were seldom researched [58].
- Developing innovative bio-sensors that depend upon the enzymes, like the aldehyde dehydrogenase or heme-containing enzyme types seem to have stagnated and lost interest in past decade, potentially due to difficulties related to the prices, commercial enzymes' unavailability, co-factor addition, or unfavorable equilibrium of enzymatic reactions or the low selectivity at same time, new enzymes are studied continuously as analytical aims for detecting of inhibiting pesticides in the assays which could be adapted simply to the bio-sensor formats, e.g., inhibition of the hydroxy-phenylpyruvate dioxygenase, which has been employed lately in bio-sensor for the β-triketone herbicides. The progression in solar cell development had resulted in enriching potentials for the sufficient wiring of the photo-synthetic enzymes on a variety of the surfaces, which open new ways for developing bio-sensors for the photosynthesis-inhibiting herbicides [57].
- In the case of the immunosensors, 2 different approaches could be followed for the purpose of achieving sensing surface renewal:(a) Ab–Ag bond breakage and re-using immunologic reagent that has been immobilized in solid phase; (b) eliminating Ag-Ab complex from solid support and immobilizing fresh immunologic materials [25]. In former approach, a thorough selection of dissociating agents has to be made for efficient dissociation of Ag-Ab complex with no impact on the bonds of association between support matrix and Ab. On developing immuno-sensor, for OP pesticide ethyl parathion utilizing ethyl parathion anti-body, a variety of the dissociating agents have been utilized [59]. Results that have been presented in the present research have indicated that the glycine-HCl (pH 2.30) buffer, which contains 1% dimethyl sulphoxide is a considerably sufficient buffer of dissociation. In the other approach, the full elimination of proteic material from surface has been accomplished in the case of the use of multiple solutions of regeneration with very high values of pH and/or high concentration levels of the salts [25,60].
- The miniaturization has been expected to have considerable impacts upon biosensensor development and applications. Bio-sensor miniaturization reduces sample volume and detection device size, as well as integrating all analytical process steps into single-sensor device. Which is why, it leads to reducing analysis cost and time [12].
- Sensor output weak electrical signal, get accurate signal represents the premise for obtaining precise results of the detection. In actual detection, there are different interfering factors, which include samples and detection environment, which is why, the signal processing and anti-interference design is detrimental for the accuracy of the results. Presently, the study on the electro-chemical modulation of sensors, weak signal processing and sample pretreatment will be effective for the

improvement of detection repeatability and stability [12].

## Conclusions

The present paper includes a review of the latest literatures about the biosensor-based instrument development for detection of the pesticide residues. Instruments that are based upon bio-receptors and fluorescence represent strong contenders for the screening of the pesticide residues and they keep becoming more and more relevant in the food and environment analyses. The bio-sensor-based instruments are still the preferable approach for qualitatively and quantitatively detecting the pesticides. In comparison with the chromatography and other approaches, the benefits of the bio-sensor equipment may be described as: in comparison to the conventional analytical tools, like the GC, a. bio-sensor-based tools are sensitive and selective for detections of pesticides. b. bio-sensor-based instruments have been easy to operate with less time and lower cost. c. following additional design and improvements, they may be utilized for the field detection and data sharing detection. All of those have shown that the biosensor-based tools have big potentials for the developments. None-the-less, a small number of the bio-sensor instruments are presently available commercially and they are yet to be established as routine or research tools, as a result of the fact that those bio-sensor instruments remain facing numerous challenges that hinder their actual applications.

a. The bio-sensor-based instruments' detection principles have been based upon specific recognitions of the biological elements, which are simple to lose activities in actual environment of detection.
b. Results of the detection are found through the electro-chemical analytical instruments and biosensor-based instruments lack complete and matched systems of analysis.
c. The majority of bio-sensor-based instruments remain at lab research stages and require the improvement of actual detection's stability and accuracy. The biosensor-based instrument application to detection of the pesticides in a variety of the agricultural products like the fruits and vegetables will be investigated additionally in future.

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## Data available Statement

The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request."

## References

World Health Organization. Human Biomonitoring: Facts and Figures; WHO Regional Office for Europe: Copenhagen, Denmark, 2015.

WHO (World Health Organization). Available online: https://www.who.int/ (accessed on 30 December 2019).

United States Environmental Protection Agency (US EPA). Available online: https://www.epa.gov/ (accessed on 30 December 2019).

Human Resources Council. United Nations General Assembly. Report of the Special Rapporteur on the Right to food. Available online: https://www.ohchr.org/en/issues/food/pages/foodindex.aspx (accessed on 30 December 2019)

Grimalt, S.; Dehouck, P. Review of analytical methods for the determination of pesticide residues in grapes. J. Chromatogr. A 2016, 1433, 1–23.

Huang, Y.; Shi, T.; Luo, X.; Xiong, H.; Min, F.; Chen, Y. Determination of multi-pesticide residues in green tea With a modi fi ed QuEChERS protocol coupled to HPLC-MS/MS. Food Chem. 2019, 275: 255–264

Caso, E.L.; Plaguicidas, C.D.E.L.O.S. Análisis de la directiva europea 98/83/CE: Paradigma de la justificacióny establecimiento de los valores paramétricos. El caso concreto de los plaguicidas. 2012, 86: 21–35.

Yan, M.; Zang, D.; Ge, S.; Ge, L.; Yu, J. A disposable electrochemical immunosensor based on carbon Screen-printed electrodes for the detection of prostate specific antigen. Biosens. Bioelectron. 2012, 38: 355–361.

Chauhan, N.; Narang, J.; Jain, U. Amperometric acetylcholinesterase biosensor for pesticides monitoring Utilising iron oxide nanoparticles and poly(indole-5-carboxylic acid). J. Exp. Nanosci. 2016, 11: 111–122.

Jaffrezic-Renault, N. New Trends in Biosensors for Organophosphorus Pesticides. Sensors 2009, 1, 2, 2001: 60-74.

Ronkainen, N.J.; H. B. Halsall, H.B. W. R. Heineman, W.R. Electrochemical Biosensors. Chemical Society Reviews 2010, 39, 11:1747-1763.

Zhao, G.; Wang, H.; Liu. G. Advances in Biosensor-Based Instruments for Pesticide Residues Rapid Detection.Int. J. Electrochem. Sci. 2015, 10.

Sun, X.; Liu, B.; Xia, K. A Sensitive and Regenerable Biosensor for Organophosphate Pesticide Based on Self Assembled Multilayer Film with CdTe as Fluorescence Probe. Luminescence 2011, 26, 6: 616-621.

Oliveira, A.C.; and Mascaro, L.H. Evaluation of Acetylcholinesterase Biosensor Based on Carbon Nanotube Paste in the Determination of Chlorphe nvinphos. International Journal of Analytical Chemistry 2011, Article ID 974216.

Sun, X.; and Wang, X. Acetylcholinesterase Biosensor Based on Prussian Blue-Modified Electrode for Detecting Organop hosphorous Pesticides. Biosensors and Bioelectronics 2010, 25, 1-2:2611-2614.

Sassolas, A.; Prieto-Simón, B.; Marty, J. Biosensors for Pesticide Detection: New Trends. American Journal of Analytical Chemistry 2012, 3: 210-232.

Yu, G.; Wu, W.; Zhao, Q.; Wei, X.; Lu, Q. Efficient immobilization of acetylcholinesterase onto amino functionalized carbon nanotubes for the fabrication of high sensitive organophosphorus pesticides biosensors. Biosens Bioelectron. 2015, 15, 68:288-294.

Jain, M.; Yadav, P.; Joshi, A.; Kodgire, P. Advances in detection of hazardous organophosphorus compounds using organophosphorus hydrolase based biosensors. Crit Rev Toxicol. 2019, 49, 5:387-410.

Senbua, W.; Mearnchu, J.; Wichitwechkarn, J. Easy-to-use and reliable absorbance-based MPH-GST biosensor for the detection of methyl parathion pesticide. Biotechnol Rep (Amst) 2020, 23,27:e00495.

Rapichai, W.; Chaichalerm, S.; Mearnchu, J.; Wichitwechkarn, J. MPH-GST sensing microplate for easy detection of organophosphate insecticides. Biotechnol Lett. 2021, 43,4:933-944.

Lan, W.; Chen, G.; Cui, F.; Tan, F.; Liu, R.; Yushupujiang, M. Development of a novel optical biosensor for detection of organophosphorus pesticides based on methyl parathion hydrolase immobilized by metal-chelate affinity. Sensors (Basel), 2012,12,7:8477-90.

Shah, M.M.; Ren, W.; Irudayaraj, J.; Sajini, A.A.; Ali, M.I.; Ahmad, B. Colorimetric Detection of Organophosphate Pesticides Based on Acetylcholinesterase and Cysteamine Capped Gold Nanoparticles as Nanozyme. Sensors (Basel), 2021,21,23:8050.

Alonso, G.A.; Istamboulie, G.; Noguer, T.; Marty, J.-L.; Munoz, R. Rapid determination of pesticide mixtures using ˜disposable biosensors based on genetically modified enzymes and artificial neural networks. Sensors and Actuators B, 2012, 164, 1: 22–28.

Amine, A.; Mohammadi, H.; Bouais, I.; Palleschi, G. Enzyme inhibition-based biosensors for food safety and environmental monitoring. Biosens. Bioelectron, 2006, 21, 1405-23.

Gamal A. E. Mostafa .Electrochemical Biosensors for the Detection of Pesticides. The Open Electrochemistry Journal, 2010, 2, 22-42.

Dan, D.; Jiawang, D.; Jie, C.; Jianming, Z.; Li, L. In situ electrodeposited nanoparticles for facilitating electron transfer across selfassembled monolayers in biosensor design. Talanta, 2008, 74, 1337-43.

Kurbanoglu, S.; Ozkan, S.A.; Merkoçi, A. Nanomaterials-based enzyme electrochemical biosensors operating Through inhibition for biosensing applications. Biosens. Bioelectron. 2017, 89, 886–898.

Dou, J.; Fan, F.; Ding, A.; Cheng, L.; Sekar, R.; Wang, H.; Li, S. A screen-printed, amperometric biosensor for the determination of organophosphorus pesticides in water samples. J. Environ. Sci. 2012, 24, 956–962.

Jaiswal, N.; Tiwari, I.; Foster, C.W.; Banks, C.E. Highly sensitive amperometric sensing of nitrite utilizing bulk-modified MnO2 decorated Graphene oxide nanocomposite screen-printed electrodes. Electrochim. Acta 2017, 227, 255–266.

Thunkhamrak, C.; Chuntib, P.; Ounnunkad, K.; Banet, P. Highly sensitive voltammetric immunosensor for the detection of prostate specific antigen based on silver nanoprobe assisted graphene oxide modified screen printed carbon electrode. Talanta 2020, 208, 120389.

Songa, E.A.; Okonkwo, J.O. Talanta Recent approaches to improving selectivity and sensitivity of Enzyme-based biosensors for organophosphorus pesticides: A review. Talanta 2016, 155, 289–304

Songa, E.A.; Arotiba, O.A.; Owino, J.H.; Jahed, N.; Baker, P.G.; Iwuoha, E.I. Electrochemical detection of glyphosate herbicide using horseradish peroxidase immobilized on sulfonated polymer matrix. Bioelectrochemistry 2009, 75, 117–123.

Zhang, W.-J.; Li, D.; Xu, Y.; Jiang, Z.; Chen, Y.; Wang, P. Synthesis and Application of Novel Molecularly Imprinted Solid Phase Extraction Materials Based on Carbon Nanotubes for Determination of Carbofuran In Human Serum by High Performance Liquid Chromatography. J. Agric. Food Chem. 2019, 67, 5105–5112.

Montes, R.; Céspedes, F.; Gabriel, D.; Baeza, M. Electrochemical Biosensor Based on Optimized Biocomposite For Organophosphorus and Carbamates Pesticides Detection. J. Nanomater. 2018, 2018, 1–13.

Dhull, V.; Gahlaut, A.; Dilbaghi, N.; Hooda, V. Acetylcholinesterase Biosensors for Electrochemical Detection of Organophosphorus Compounds: A Review. Biochemistry Research International. Volume 2013, Article ID 731501.

Weinbroum, A.A. Pathophysiological and clinical aspects of combat anticholinesterase poisoning. British Medical Bulletin 2004, 72: 119–133.

Pope, C.; Karanth, S.; Liu, J. Pharmacology and toxicology of cholinesterase inhibitors: uses and misuses of a common Mechanism of action. Environmental Toxicology and Pharmacology 2005, 19, 3:433–446.

Andriukonis, E.; Celiesiute-Germaniene, R.; Ramanavicius, S.; Viter, R.; Ramanavicius, A. From Microorganism-Based Amperometric Biosensors towards Microbial Fuel Cells. Sensors 2021, 21, 2442.

Lei, Y.; Mulchandani, P.; Wang, J.; Chen, W.; Chen, W.; Mulchandani, A. Highly sensitive and selective amperometric microbial Biosensor for direct determination of p-nitrophenyl-substituted Organophosphate nerve agents. Environ. Sci. Technol., 2005, 39: 8853-57.

Chouteau, C.; Dzyadevych, S.; Durrieu, C.; Chovelon, J. M. A bienzymatic whole cell conductometric biosensor for biosensor for heavy metal ions and pesticides detection in water samples. Biosens. Bioelectron, 2005, 21:273-81.

Mulchandani, P.; Chen, W.; Mulchandani, A.; Wang, J.; Chen, L. Amperometric microbial biosensor for direct determination of or- ganophosphate pesticides using recombinant microorganism with surface expressed organophosphorus hydrolase. Biosens. Bioelectron, 2001, 16: 433-37.

Grawe, G.F.; de Oliveira, T.R.; de Andrade Narciso, E.; Moccelini, S.K.; Terezo, A.J.; Soares, M.A.; Castilho, M. Electrochemical biosensor for carbofuran pesticide based on esterases from eupenicillium shearii FREI-39 Endophytic fungus. Biosens. Bioelectron. 2015, 63, 407–413.

Priti, M.; Wilfred, C.; Ashok, M. Microbial biosensor for direct determination of nitrophenyl-substituted organophosphate nerve agents using genetically Moraxella sp. Anal. Chim. Acta, 2006, 568, 217-221.

Wang, J. Electrochemical nucleic acid biosensors. Anal. Chim. Acta, 2002, 569, 63-71.

Lucrelli, F.; Kicela, A.; Palchetti, G.; Marrazza, G.; Mascini, M. Electrochemical DNA biosensor for analysis of wastewatersamples. Bioelectrochemistry, 2002, 58, 113-118.

Arora, K.; Chaubey, A.; Singhal. R.; Singh, R.P.; Samanta, S.B.; Chand, S.; Malhotra, B.D. Application of electrochemically prepared polypyrrole-polyvinyl sulphonate films to DNA biosensors. Biosens. Bioelectron., 2006, 21: 1777-83.

He, P.G.; Xu, Y.; Fang, Y.Z. A review: electrochemical DNA biosensors for sequence recognition. Anal. Lett., 2005, 38: 2597-2623.

Nirmal, P.; Kavita, A.; Surinder, P. S.; Manoj, K. P.; Harpal, S.; Bansi, D. M. Polypyrrole-polyvinyl sulphonate film based disposable nucleic acid biosensor. Anal. Chim. Acta, 2007, 589: 6-13.

Nirmal, P.; Sumana, G.; Kavita, A.; Harpal, S.; Malhotra, B.D. Improved electrochemical nucleic acid biosensor based on polyaniline-polyvinyl sulphonate. Talanta, 2008, 74: 1337-43.

Pérez-Fernández, B.; Mercader, J.V.; Checa-Orrego, B.I.; de la Escosura-Muñiz,A.; Costa-García, A. A monoclonal antibody-based immunosensor for the Electrochemical detection of imidacloprid pesticide, Analyst 2019, 144. Doi:10.1039/c9an00176j.

Hleli, S.; Martelet, C.; Abdelghani, A. Atrazine analysis using an Impedimetric immunosensor based on mixed biotinylated selfassembled monolayer. Sens. Actuators B, 2006, 113: 711-17.

Wei, W.; Zong, X.; Wang, X.; Yin, L.; Pu, Y.; Liu, S. A disposable amperometric Immunosensor for chlorpyrifos-methyl based on immunogen / platinum doped Silica sol – gel film modified screen-printed carbon electrode, Food Chem. 2012, 135: 888–892.

Romero, V.; Vila, V.; de la Calle, I.; Lavilla, I.; Bendicho, C. Turn–on fluorescent sensor for the detection of periodate anion following photochemical synthesis of nitrogen and sulphur co–doped carbon dots from vegetables. Sensors Actuators B Chem. 2019, 280:290–7.

Chang, M.M.; Ginjom, I.R.; Ng, S.M. Single-shot 'turn-off' optical probe for rapid detection of paraoxon-ethyl pesticide on vegetable utilising fluorescence carbon dots. Sensors Actuators B Chem. 2017, 15,242, 22:1050–6.

Fatahi, Z.; Esfandiari, N.; Ehtesabi, H.; Bagheri, Z.; Tavana, H.; Ranjbar, Z. Physicochemical and cytotoxicity analysis of green synthesis carbon dots for cell imaging. EXCLI J 2019, 27, 18:454–66.

Hou, J.; Dong, J.; Zhu, H.; Teng, X.; Ai, S.; Mang, M. A simple and sensitive fluorescent sensor for methyl parathion based on l -tyrosine methyl ester functionalized carbon dots. Biosens Bioelectron [Internet]. 2015. June;68:20–6.

Rocaboy-Faquet, E.; Barthelmebs, L.; Calas-Blanchard, C.; Noguer, T. A novel amperometric biosensor for ß-triketone herbicides based on hydroxyphenylpyruvate dioxygenase inhibition: A case study for sulcotrione. Talanta 2016, 146, 510–516.

Bucur, B.; Munteanu, F.; Marty, J.; Vasilescu, A. Advances in Enzyme-Based Biosensors for Pesticide Detection Biosensors 2018, 8, 27; doi:10.3390/bios8020027.

Kandimalla, V.B.; Neeta, N.S.; Karanth, N.G.; Thakur, M.S ;. Roshini, K.R.; Rani, B.E.A.; Pash, A.; Karanth, N.G.K. Regeneration of ethyl parathion antibodies for repeated use in immunosensor: a study on dissociation of antigens from antibodies. Biosens. Bioelectron, 2004, 20: 903-06.

Sardinha, J.P.M.; Gil, M.H.; Mercader, J.V.; Montoya, A. Enzymelinked immunofiltration assay used in the screening of solid supports and immunoreagents for the development of an azinphosmethyl flow immunosensor. J. Immunol. Methods, 2002, 260: 173-182.