In the recent years, environmental pollution has become a major global concern due to its adverse effects on human health. This environment contamination is primarily attributed to the extensive use of organo-phosphorus pesticides in agriculture and public health pest control. These toxic agents are affecting air and soil, as well as water resources. Therefore, rapid, sensitive, selective and reliable detection of these toxic compounds is crucial. Many biosensors based on various emergent nano-structured materials have been manufactured for this purpose. Despite their acceptable sensitivity level and detection limits, these nano-assembled biosensors are tested under controlled laboratory conditions, usually at room temperature. Therefore, they might not be accurate for on-line detection of pesticides in arid areas where temperature fluctuations are significant. This work provides a brief review of scientific achievements in this field and gives a comprehensive comparison between different technologies presently in use.

**Key words:** Enzyme; Nano-Sensor; Neurotoxins; High Sensitivity.

To prevent insects caused damage and to increase agricultural yield, pesticides are used all over the world (Gámiz et al. 2016; Hussain et al. 2015; Nassar 2015). However, pesticide residue can have adverse effects on human health (Magos 1992) due to the inhibition of the human enzymatic activity like Acetyl-Cholinesterase (AChE), which is the primary enzyme in the body in charge of proper function of the human brain and central nervous system (Doctor and Saxena, 2005; Cole et al. 2005). Therefore, detection and monitoring of organo-phosphorus (OP) neurotoxin residues, which is the most commonly used pesticide, is essential to protect environmental resources and supplies (Rajani, 2015; Jepson et al. 2014; Bottoni et al. 2013). Given the significance of the topic, extensive studies are being carried out in a search for novel OP neurotoxin detection methods that are more rapid, sensitive, selective, reversible and reliable in comparison with traditional techniques based on chromatography and mass spectroscopy employed in slow and expensive off-site experiments (Mahajan and Shrivastava 2005; Krock and Wilkins, 1996).

Graphene is a new material that has captured the attention of scientific community as a two-dimensional carbon material (Tewary and Zhang, 2015; Tanaka and Iijima, 2014; Warner et al. 2013) is characterized by amazing thermal and mechanical properties, in addition to its large surface area and excellent optical and electrical signal propagation (Tewary and Zhang, 2015; Tanaka and Iijima, 2014; Warner et al. 2013). These exceptional proprieties make graphene a candidate for sensor platform’s transducer integration. Recently, many research efforts have been focused on the fabrication of an enzyme graphene hybrid sensor (Zhou, Zhai, and Dong 2009; Shan 2010; Park et al. 2011; Zheng et al. 2014). Detection techniques can be either based on activity inhibition of different enzymes abundantly present in pesticides (AChE, BChE, Tyr, etc.) (Liu et al. 2011; Zhai et al. 2014), or on the catalytic activity of organophosphorus hydrolase (OPH) (Park et al. 2011; Yang et al. 2011). OPH is a well-characterized metallo-enzyme originally extracted from *Pseudomonas diminuta* bacteria (Heo et al. 2003) or *Escherichia coli* bacteria (Raimondi et al. 2003) exhibiting an aptitude to hydrolase a large variety of OP pesticides and neurotoxins. Despite the promise of immunoassay techniques, inhibition-mode based sensors are unsuitable for detoxification processes due to their poor selectivity, protracted analysis and irreversibility. The latter is due to the fact that the enzyme allows one-time inhibition process and should be regenerated before reuse (Rogers et al. 1999). Catalytic receptor enzyme based sensors can detect OP pesticide traces by monitoring pH changes in the tested environment resulting from enzymatic hydrolase activity. Recently, many enzyme based sensors have been manufactured for the detection of several neurotoxin analyte models, including paraxon (Takruni, Almuaibed, and Townshend 1993), methyl parathion (Ng et al. 2015), carbaryl (Rogers et al. 1999), and fenithion (Liu et al. 2014; Li et al. 2014).

These sensors showed a wide detection linear range with a very promising detection limit in the pico-mole range [Ng et al., 2015; Park et al., 2011 and Raimondi, 2003]. Dong et al.
(2015) from Shandong Agricultural University, China, have demonstrated an oxime-sensor based on oxime that functions by dispersing prali-doxime (PAM) on graphene quantum dots (GQDs) modified glassy carbon electrode for fenthion neurotoxin detection, which is a non-electroactive OP pesticides. This sensor was capable of in the 0.1pM-50 µM range, with the detection limit of 6.8pM (Rajani, 2015; Roger et al., 1999; Shan et al., 2010 and Sheshink, 1997). However, these sensors were fully tested and characterized under room temperature conditions (25±2°C), which limits their application for on-site detection, especially in arid areas, due to the poor thermal stability of the enzymes (Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, 7 Volume Set 2010), which possess broadened working temperature ranges.

Table 1 summarizes the different active ingredients in internationally commercialized and used OP pesticides, along with their names, chemical formulas, Van Der Waals sphere surface and maximum lethal concentration 50 (LC50), ranked by their toxicity to humans and animals. In fact, pesticides with the lowest LC50 values are the most toxic to humans in the case of acute exposure, assuming oral exposure route.

Despite enhancements in sensitivity and detection limits, as these nano-assembled biosensors are tested under room temperature operating conditions (25±2°C), their direct use in arid environments and high temperature ranges is limited. This sensing limitation is essentially due the low thermal stability of the enzyme because of its denaturation at high temperature ranges (Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, 7 Volume Set 2010).

ORGANO-PHOSPHORUS PESTICIDE DETECTION:
Organophosphorus (OP) compounds were widely used in agriculture as insecticides when they replaced the toxic organo-chlorinated compounds, such as dichlorodiphenyl trichloroethane (DDT), which was banned in the 70s because of its suspected carcinogenic properties and persistence in the environment (Gaspard et al. 2015). Even though OP-based pesticides have limited persistence in the environment due to their relatively rapid biodegradation, they are responsible for insidious poisoning because of their large-scale presence in the environment. In 2007, the United State Environmental Protection Agency (US EPA) (EPA n.d.) reported that about 15,000 tons of OPs were used as insecticides in the United States, 80% which was used in agriculture (US EPA n.d.).

Several recent environmental investigations (London et al. 2002; Sheshinski 1997; Marsh et al. 1996) have indicated that malathion (C6H11O2PS - CAS Number 121-75-5), diethyl parathion (C12H18NO3PS-CAS Number 56-38-2), parathion methyl((CH3)2OP(S)OCH3 - CAS Number 298-00-0), and diazinon (C12H19NO3PS - CAS Number 333-41-5) are among the most widely used organophosphorus insecticides. Since 1950, more than 100,000 different OPs have been synthesized and over 350 have received authorization to be commercialized [(US EPA n.d.). According to the World Health Organization (WHO), OP-based insecticides are the greatest environmental pollutants. It is estimated that, annually, they cause more than 3 million poisonings, suicidal or accidental, leading to over 300,000 deaths (Kasner et al. 2012). Upon suicidal or accidental exposure to organophosphorus (OP) pesticides, neurotoxin agents can circulate freely in the human bloodstream and can rapidly spread to vital organs, i.e., heart, liver, kidney, lungs and brain, causing inhibition of acetylcholinesterase (AChE), which is a key enzyme in the transmission of nerve messages (Doctor and Saxena, 2005; Cole et al. 2005).

For prevention and reduction of human exposure to these hazardous OP pesticides, considerable progress has been made by the international scientific community in the development of rapid and reliable sensors, helping in the detection and monitoring of OP pesticides in the environment (Kapka-Skrzypczak et al. 2011; Kumar et al., 2015; Marsh et al. 1996). The following section gives a brief overview of the currently utilized enzyme based techniques and emergent materials.

ENZYMATIC NANO-SENSORS FOR OP PESTICIDE DETECTION: About 1,653 scientific publications and 4 international patents related to OP pesticide detection techniques are recorded between 1990 and 2017 by Elsevier’s bibliographic database “Scopus”. US and Chinese research teams have produced about 42% of these publications. Their review indicates that the most sensitive and competitive sensors are synthesized by using novel nanostructured materials, such as carbon nanotubes (CNT), quantum dots (QD) or graphene nano-sheets (GRs). This is essentially due to their exceptional thermal, mechanical and electrical proprieties.
Table 1: OP pesticide active ingredient names, chemical formula, Van Der Waals sphere surface and lethal concentration 50 (LC$_{50}$) as indicated by The Occupational Safety and Health Administration (OSHA)

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Chemical Formula</th>
<th>Structural Formula</th>
<th>LC$_{50}$ (mg·kg$^{-1}$) (oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbufos</td>
<td>C$_9$H$_2$O$_2$PS$_3$</td>
<td><img src="image1" alt="Structural Formula" /></td>
<td>4.5</td>
</tr>
<tr>
<td>Azinphos-Methyl</td>
<td>C$_{10}$H$_6$N$_3$O$_3$PS$_2$</td>
<td><img src="image2" alt="Structural Formula" /></td>
<td>5–20</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>(CH$_3$O)$_3$PS(OCC$_6$H$_4$NO$_2$)</td>
<td><img src="image3" alt="Structural Formula" /></td>
<td>6</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>C$<em>{17}$H$</em>{27}$NO$_3$PS</td>
<td><img src="image4" alt="Structural Formula" /></td>
<td>92–276</td>
</tr>
<tr>
<td>Phosmet</td>
<td>C$<em>{11}$H$</em>{29}$NO$_3$PS</td>
<td><img src="image5" alt="Structural Formula" /></td>
<td>147–316</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>C$<em>{12}$H$</em>{22}$NO$_3$PS</td>
<td><img src="image6" alt="Structural Formula" /></td>
<td>235</td>
</tr>
<tr>
<td>Diazinon</td>
<td>C$<em>{12}$H$</em>{21}$N$_2$O$_3$PS</td>
<td><img src="image7" alt="Structural Formula" /></td>
<td>300–400</td>
</tr>
<tr>
<td>Acephate</td>
<td>C$<em>{14}$H$</em>{25}$NO$_3$PS</td>
<td><img src="image8" alt="Structural Formula" /></td>
<td>980</td>
</tr>
<tr>
<td>Malathion</td>
<td>C$<em>{10}$H$</em>{19}$O$_6$PS</td>
<td><img src="image9" alt="Structural Formula" /></td>
<td>5,500</td>
</tr>
</tbody>
</table>

Fig. 1: Cooling Degree Days (CDD) for agricultural sites in Saudi Arabia; i.e. Al Jawf, Tabuk, Wadi Ad-Dawasir (Ar’ Riadh) and Sabya (Jiazan) areas.
Fig. 2: Schema of the diazinon hydrolyze chemical reaction with OPH enzyme.

Table 2: Analytical characteristics of some enzymatic nano-sensors for OP pesticide detection

<table>
<thead>
<tr>
<th>Sensor’s configuration</th>
<th>OP detected model</th>
<th>Enzyme</th>
<th>Linear Range</th>
<th>DL (pM)</th>
<th>Ref</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE-SiSG-AuNPs</td>
<td>Monocrotophos</td>
<td>AChE</td>
<td>10 – 1000 M</td>
<td>0.6 ng mL⁻¹</td>
<td>47</td>
<td>2007</td>
</tr>
<tr>
<td>SPE with ZnO NPs and quantum dots (ZnS@CdS, QDs)</td>
<td>Paraoxon</td>
<td>AChE</td>
<td>10 pM – 4 nM</td>
<td>8.0 pM</td>
<td>50</td>
<td>2008</td>
</tr>
<tr>
<td>CS²-AuNPs-OPH conjugated with 7-Diethylamino-4-methylcoumarin</td>
<td>Paraoxon</td>
<td>OPHEC 3,1 &amp; 1</td>
<td>NR</td>
<td>5 x 10 pM</td>
<td>48</td>
<td>2015</td>
</tr>
<tr>
<td>GO³-CN² composite film</td>
<td>carbaryl/trichlorfon</td>
<td>AChE</td>
<td>10 nM – 100 nM</td>
<td>2.5 nM</td>
<td>49</td>
<td>2015</td>
</tr>
<tr>
<td>trichlorfon</td>
<td>10 nM – 60 nM</td>
<td>1.2 nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERGO²-GCE⁴-AuNPs-β-CD³-PB-CS⁵</td>
<td>Malathion</td>
<td>AChE</td>
<td>7.98 pg mL⁻¹ – 2.00 x 10⁷ pg mL⁻¹</td>
<td>4.14 pg mL⁻¹</td>
<td>48</td>
<td>2015</td>
</tr>
<tr>
<td>carbaryl</td>
<td>4.3 pg mL⁻¹ – 1.00 x 10⁷ pg mL⁻¹</td>
<td>1.15 pg mL⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCE⁴-poly(FBThF⁵)-f-MNPs²/AChE biofilm</td>
<td>Paraoxon</td>
<td>AChE</td>
<td>0.0125 mM – 2.6 mM</td>
<td>6.66 x 10⁻⁷ mM</td>
<td>51</td>
<td>2016</td>
</tr>
</tbody>
</table>

NR: not reported

¹SiSG: Silica Sol-Gel

²CS: Chitosan

³SPE: Screen Printed Electrode

⁴FBThF: 4,7-di(furan-2-yl)benzo[c][1,2,5]-thiadiazole

⁵f-MNPs: Carboxyl Group Modified Magnetic Nanoparticles

⁶ERGO: Electrochemical Reduced Graphene Oxide

⁷β-CD: β-cyclodextrin

⁸PB-CS: Prussian blue-chitosan

⁹GCE: Glass Carbon Electrode

¹⁰AChE: Acetylcholinesterase

Graphene remains the most competitive nanomaterial, as it offers the best surface area in addition to adequate electrical signal amplification. However, only 30 publications are recorded in “Scopus” database in which authors discuss graphene based biosensors for OP pesticide detection. Wang et al. (2011) have synthesized a nano-hybrid gold nano-particles (Au-NPs) reduced graphene oxide nano-sheets (crGRs) enzymatic biosensor based on AChE. Ultrasensitive detection of paraoxon neurotoxin was achieved with the limit of detection (DL) of 0.1 pM. According to the authors, very low DL is due to the nano-assembly concept, which combines advantages of metal nanoparticles, carbon nano-sheets and self-assembly techniques. Haiyan Zhao et al. (2015) have fabricated an AuNPs / crGRs / β-cyclodextrin (β-CD) and Prussian blue-chitosan (PB-CS) AChE based biosensor. The inhibition of the AChE enzymatic activity was realized by the target analytes malathion and carbaryl. This sensor showed wide linear ranges of 7.98-2.00 x 10⁷ pg mL⁻¹ and 4.3-1.00 x 10⁷ pg mL⁻¹ with low DL of 4.14
pg mL\(^{-1}\) and 1.15 pg mL\(^{-1}\) for malathion and carbaryl, respectively. Table 2 shows the analytical characteristics of some enzymatic nano-sensors for OP pesticide detection, i.e., linear range and the detection limit. It is clear that different materials and sensor configurations have been synthesized, such as nanoparticles (zirconium dioxide, gold nanoparticles), graphene oxide, quantum dots, polymer, chitosan and silica in order to enhance the sensor sensitivity performance.

Despite enhancements in sensitivity and detection limits, as these nano-assembled biosensors are tested under room temperature operating conditions (25±2°C), their direct use in arid environments and high temperature ranges is limited. This sensing limitation is essentially due the low thermal stability of the enzyme because of its denaturation at high temperature ranges (Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, 2010).

**OP detection in arid regions:** A study (Hegazy and Kamel 2015) was conducted from 2009 to 2011 in Saudi Arabia, focusing on the acute organophosphorus poisoning (OPP) patterning in patients admitted to Al-Dammam PCC (Poison Control Centre). The authors reported that about 40% of the accidental contamination cases had occurred in July because of the increase in agricultural activities and the wide use of OP pesticides (Güloglu and Kara 2005; Mert and Bilgin 2006). Al-Hadhrami et al. (2013) recently conducted a comprehensive review of the heating and cooling patterns in the Kingdom. Provided annual cooling degree-days (CDDs) values was 4128, 4359, 5555, 5774 in Al-Jawf, Tabuk, Wadi Ad-Dawasir (ArRiyad province) and Sabya (Jazan province) areas respectively. These high CDD values indicate that these areas are characterized by large temperature fluctuations, which are particularly pronounced in summer time. On the other hand, these Saudi areas are also known for extensive agricultural activity ("FAO Country Profiles" n.d.). Hence, these areas may form real OP pesticide contamination sites. This was confirmed by Al-Hatim et al. (2015) by collecting and analyzing samples from 3 dams and 44 wells in Jazan province (including Sabya’s area) for the detection of 15 different types of used insecticides. The analysis results indicated that the most common OP pesticide trace in both surface water and groundwater samples was diazinon (C\(_{12}\)H\(_{21}\)N\(_2\)O\(_3\)PS-CAS Number 333-41-5) with an average concentration in the 0.098-0.117 µg L\(^{-1}\) range. Diazinon is mobile and moderately persistent in the environment (US EPA n.d.). Hence, additional efforts are necessary to synthesize a novel enzymatic nano-assembly biosensor suitable for reliable on-site detection of diazinon target analyte. For diazinon detection, OPH enzyme can be an interesting candidate for detecting such neurotoxin traces even in very low quantities (Fig. 3). On-site extracted OPH enzyme form *Escherichia coli* bacteria can be used to address this issue and to enhance the sensor performance, especially in arid areas.

**CONCLUSION**

This work provides a brief review of the recent developments in the enzymatic nano-material research and resulting hybrid sensors for organo-phosphate neurotoxin residue detection. While very promising sensitivity capabilities have been achieved, additional efforts are needed for developing biosensors suitable for reliable on-site detection and capable of operating in arid areas, as temperature fluctuations can cause the enzyme denaturation and alteration, preventing it from performing its function, i.e., the hydrolyze chemical reaction.

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ENZYMATIC NANO-MATERIAL BASED HYBRID SENSORS FOR ORGANO-PHOSPHATE NEUROTOXIN RESIDUE


