Review Article

Revolutionizing Food Safety and Crop Production: Harnessing Novel Biosensors

Abstract

Farmers and food manufacturers are under immense pressure from consumers and food safety regulations to deliver pollutant-free, high-quality foods. The extensive use of chemicals in food production poses ecological as well as health risks. In order to meet the demand for safe, preservative-free foods, rapid sensing techniques are required. Traditional analysis methods are time consuming, laboratory bound, expensive and require highly skilled personnel. Alternative analysis systems such as biosensors, which are user-friendly and enable real-time monitoring in the field should also be used. Biosensors have been developed to detect foodborne pathogens such as *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes*, etc. that cause food contamination, and a large number of cases are reported annually. Mycotoxin-contaminated food presents a serious threat to food safety. Biosensors have been utilised to identify Penicillium and aflatoxin infections, which are major mycotoxins found in food. Additionally, biosensors for identifying artificially ripened fruits have also been developed. Biosensors have been developed to detect pesticide residues such as atrazine, glyphosate, 2,4-D, methyl parathion, lindane, etc. Early identification of plant pathogens, including bacteria, viruses, and fungi, is crucial because it enables farmers to take the necessary precautions to control the disease. Pathogens such as *Fusarium* sp., *Phytophthora palmivora*, tomato leaf curl virus are among some of the pathogens that have been successfully detected using novel biosensors. They can also be used for detecting heavy metals as they are cheaper, faster, more reliable and selective than traditional analysis methods. A bacterial biosensor was developed using *Bacillus megaterium*, which was sensitive to heavy metals like cadmium, copper and zinc in soil. Additionally, biosensors have been developed to detect heavy metal pollution in plants as well as irrigation water.

Keywords: Biosensor, food safety, pollution, disease detection, heavy metal detection, pesticide residue.

1. Introduction

Farmers and food manufacturers are under immense pressure from consumers to provide quality foods that are free from pollutants and contaminants. Pollutants present in soil, water sources and food are a major concern for human health due to the extensive use of chemicals. The indiscriminate use of pesticides and chemicals in agriculture and food industry has led to various ecological problems as well as human and animal health hazards [1]. According to the World Health Organisation, people living in low-income regions of Africa and Southeast Asia and children under the age of five are more susceptible to foodborne illness [2]. As a result, strict regulations have been introduced to monitor and control the release of contaminants. To detect these contaminants on site, rapid sensing methods are essential. Moreover, the demand for fresh foods that are pathogen-free and contain less preservatives have also increased the demand for rapid sensing methods.

The traditional analysis methods used to detect pesticides and pollutants include high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), etc. [3]. These analysis methods can only be done in laboratories, are time consuming, expensive and require highly skilled personnel [4] as these are highly technical procedures that require proper education and training to be carried out accurately. Hence, there is an increasing need for more easy-to-use analysis methods that can be performed on-site [5]. There comes the importance of alternative analysis systems such as biosensors, which are simple and allow real time monitoring in the field.

2. Biosensor

Biosensors have been widely used for detecting chemical pollutants and food-borne pathogens. The importance of biosensors is evident from the increasing growth of the biosensor market in recent years. The biosensor market is expected to increase from $22.6 billion in 2022 to $39.2 billion by 2032. North America leads in market size, valued at over $8.6 billion in 2021, followed by Europe. The Asia-Pacific region is projected to grow the fastest, with India being the fastest-growing market within this region [6].

The International Union of Pure and Applied Chemistry (IUPAC) defines a biosensor as “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal or optical signals” [7]. Biosensors are analytical devices which incorporate a biological material or a biologically-derived material as the recognition molecule to detect contaminants. They usually produce an electric signal that is proportional to the concentration of a specific group of analytes. The commonly used recognition molecules are antibodies, aptamers, nucleic acids and enzymes.

Antibodies serve as recognition elements in immunoassays, enabling the separation of target analytes from samples. They are crucial for detecting food contaminants, including microorganisms, mycotoxins, veterinary drugs, and allergens. Another recognition element used instead of antibodies is aptamer. Aptamers are artificial DNA, RNA, peptides, etc. that bind to specific target molecules and are used as an alternative to antibodies [8]. The production of aptamers is cheaper compared to antibodies because their synthesis does not rely on animal cells [9].

Nucleic acids are widely used as specific recognition molecules in traditional DNA hybridization assays. When nucleic acids are used as recognition molecules, they require an additional step of sample preparation, which is not needed when antibodies or aptamers are used. Therefore, the use of nucleic acids in food quality assessment is rare. Nucleic acids are commonly used in biosensors to detect plant pathogens, as these are found to be more precise than other recognition molecules [10].

Enzymes are commonly used in agricultural biosensors as they can be used as target-specific recognition molecules for the detection of insecticides, herbicides and fungicides. Enzymes are preferred because they are highly stable and can be used repeatedly and continuously. They can also maintain catalytic activity and can be easily separated after complex formation [11,12].

2.1 Types of Biosensors

Some of the major types of biosensors used in agriculture are:

1. *Amperometric biosensor*: Electroactive species present in test samples are detected in these types of biosensors. Electrodes are used in these which produces current due to presence of analytes in the test samples.

2. *Potentiometric biosensor*: These biosensors also incorporate electrodes. Biochemical reactions take place in these biosensors like oxidation, reduction, etc. which helps in quantification of the substrate.

3. *Optical Biosensor*: This type of biosensor is based on the principle of optical diffraction or chemiluminescence where the luminescence or fluorescence may increase or decrease in the presence of the analyte.

4. *Calorimetric Biosensor*: catalysts are used in these biosensors, which leads to exothermic reaction and the heat can be quantified to detect the contaminant in the samples.

5. *DNA Biosensor*: Hybridization between the DNA within the biosensor and genetic material of the pathogen or contaminant will take place which can later be determined to detect the presence of the pathogenic material.

6. *Acoustic biosensor*: An acoustic biosensor is a type of biosensor that utilizes acoustic waves for the detection of biological molecules or changes in biological systems. These biosensors are based on the principle that the binding of target molecules to a surface causes measurable changes in acoustic properties.

Table 1. Types of biosensors, their advantages, disadvantages and examples

SL No.	Type of Biosensor	Advantages	Disadvantages	Example
1.	Amperometric biosensor	Suitable for mass production, most popular biosensors globally and are very sensitive	Narrow or limited temperature range, unstable current and short or limited shelf life	Glucose meters, lactate meters and alcohol breathalyzers
2.	Potentiometric biosensor	Easily fabricated in large quantites, low cost and is a simple monitoring instrument	Measurement error due to interference from other contaminants	Ion-selective electrodes, metal oxide-based biosensors and glass electrodes
3.	Optical Biosensor	Cost-effective, high sensitivity, selectivity and small size	Prone to physical changes and interference from environmental factors	surface plasmon resonance (SPR), fluorescent biosensors, refractive index and Raman scattering
4.	Calorimetric Biosensor	Scalability, Ease of use and ease of fabrication	Long experimental procedures and lack of specificity in	Thermopiles or thermistors

			temperature measurements	
5.	DNA Biosensor	High specificity, early detection and rapid results	Costly to develop and manufacture, May require extensive sample preparation, sensitivity to contamination and has limited range	Gene expression sensors, pathogen detection, food safety testing
6.	Acoustic biosensor	Small size, high sensitivity, fast detection and good frequency response,	High sensitivity to temperature, unsuitable for static environments, some crystals dissolve in water and are capable of dissolving in highly humid environments.	Piezoelectric crystal and surface acoustic devices

3. Biosensor for food quality determination

The food industry has grown rapidly over the last fifty years to meet the needs of the growing population and changing lifestyles. Due to socioeconomic and health impacts, the consumption of ready-to-eat foods makes quality control an important issue [13]. Therefore, the food industry developed strategies and technologies for rapid, sensitive, reliable, and cost-effective analytical methods to determine the presence of foodborne pathogens and contaminants [14,15]. Biosensors represent an important tool in food quality analysis.

3.1 Foodborne pathogens

Foodborne pathogens have become an important food safety concern [16]. With the improvement of living standards, consumers are becoming increasingly concerned about the food safety and nutritional quality of their diet. The commonly used methods for the detection of foodborne pathogens include culture-based method, polymerase chain reaction (PCR) and enzyme-linked immune-sorbent assay (ELISA). They take 3 to 5 days to obtain results, need well-trained technicians for complex DNA extraction procedures and lack sufficient sensitivity [17,18]. Biosensors are considered powerful analytical tools and have attracted a great deal of attention for the rapid detection of foodborne pathogens [19,20].

Among the foodborne pathogens, *Salmonella* is the major cause of foodborne disease outbreaks in humans and animals [21,22]. Fresh-cut vegetables are one of the main reservoirs of *Salmonella typhimurium* [23]. Man *et al.* [24] developed a biosensor to detect the presence of *Salmonella typhimurium* in fresh-cut vegetables. An aptamer that binds with the pathogen was used in this biosensor. On reaction with *Salmonella*, the colour of the solution, which contained the extract of the vegetable and aptamer, changed from red to shallow red. This colour change was detected using a smart phone application that was developed to identify small colour differences in the sample solution. The recoveries ranged from 91.68 % to 113.76 % for the fresh-cut vegetable samples.

Table 2. *Salmonella* detection of an aptamer-based biosensor

Sample	***Salmonella* added** (cfu ml^{-1})	***Salmonella* detected** (cfu ml^{-1})	**Recovery (%)**
1	0	Not detected	-
	0	Not detected	-
2	60	56	93.38
	60	55	91.68
3	600	557	92.85
	600	682	113.76
4	6000	6457	107.61
	6000	6761	112.68

[24]

Another important food pathogen is *Escherichia coli*, which causes life-threatening diseases such as hemorrhagic colitis, hemolytic-uremic syndrome, and severe gastrointestinal infections [25]. A biosensor developed by Gangwar *et al.* [26] was able to detect *E. coli* using an anti-*E. coli* antibody. The antibodies were placed on an electrode, and it was found that the resistance of the system increased with increasing concentrations of *E. coli*. The biosensor was also found to be very selective to *E. coli* and could be used in real-life conditions.

Listeria monocytogenes is another food pathogen with high mortality rates that causes life-threatening diseases like gastroenteritis, meningo-encephalitis and sepsis [27,28]. The World Health Organization considers *L. monocytogenes* as one of the most lethal pathogens as it can withstand high pH, high salt concentration and low-temperature conditions, resulting in 1 million cases per year in Southeast Asian countries [29]. India, being one of the largest producers of fish, has reported the presence of *Listeria monocytogenes* in fish produced from Kerala, Kashmir and the Tuticorin region [30,31,32]. A biosensor was developed that utilizes the listeriolysin O (LLO) protein, which is the primary virulence factor in *Listeria* sp. [33]. An anti-LLO antibody was used as the analyte to identify *Listeria* contamination in food samples. The antibody was placed on an electrode and it formed a complex with the LLO protein of the pathogen. The complex formation led to an increase in the resistance of the electrode surface with increase in the LLO concentration. The biosensor was also selective for *Listeria* when it was tested with water and milk samples and it could specifically distinguish *Listeria* from other pathogens.

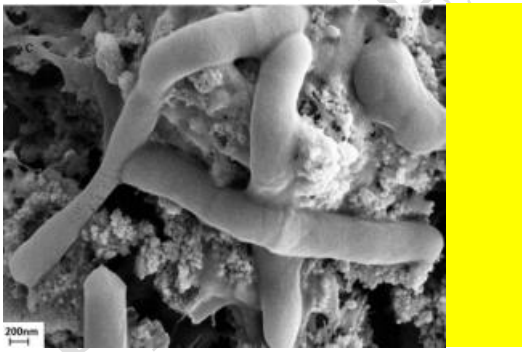

Fig.1. Anti-LLO antibody complex conjugating with *L. monocytogenes*

3.2 Mycotoxins

Food contamination by mycotoxins is a significantly growing public concern in terms of food safety and security due to morbidity, mortality and monetary loss. Among food mycotoxins, aflatoxins, produced by *Aspergillus* sp., are the principal mycotoxin with harmful impacts on human as well as animal health. Aflatoxins are listed as the most potent naturally occurring carcinogen [34]. A biosensor with aptamers and DNAzymes was developed to detect the contamination of aflatoxins in samples such as maize, rice, chilli, black pepper and groundnut [35]. DNAzymes are single-stranded catalytic DNA that are synthesised through in vitro selection processes [36]. The aptamers will bind to the aflatoxins and isolate them. DNAzymes will react to the isolated aflatoxins and produce a colour change indicating the presence of the mycotoxin.

Another fungus that deteriorates the quality of fruits is *Penicillium digitatum,* which causes green mould on citrus fruits, leading to postharvest losses of up to 30 to 50 % [37]. To decrease such losses, sensor technologies that allow early-stage fungal detection are needed to prevent further spread of the disease among stored oranges. Chalupowicz *et al.* [38] introduced a biosensor based on a genetically modified bioluminescent *Escherichia coli* strain that produces luminescence in the presence of volatile organic compounds (VOCs). These bacterial strains will detect a VOC called limonene when exposed to air released from the infected fruits. Limonene is a part of the fruits natural defense mechanism against fungal pathogens [39,40], but it facilitates infection by *Penicillium digitatum* [41]. As *Penicillium* infection increased, the limonene released by citrus fruits also increased. The *E. coli* strains can detect the increased limonene content and produce luminescence. The use of this biosensor helped to detect infection before the appearance of visible signs on the surface of citrus fruits.

Deoxynivalenol, also called vomitoxin, is one of the most common mycotoxins in cereal crops [42]. It has been listed in the prioritized chemicals of concern for human health by the European Human Biomonitoring Initiative [43,44]. It causes anorexia, diarrhoea and vomiting in humans and animals [45]. A biosensor that integrates genetically modified yeast to produce fluorescence was introduced by Yang *et al*. [46]. An antibody that binds with deoxynivalenol was used and the complex formed between them was determined by the fluorescence produced by yeast. The biosensor was tested on water and crops such as wheat, corn and fodder and the recovery ranged from 93.80 to 128 %.

Table 3. Deoxynivalenol detection of biosensor

Sample	**Deoxynivalenol added (ng ml^{-1})**	**Deoxynivalenol detected (ng ml^{-1})**	**Recovery (%)**
Wheat	1	1.22	121.70
	10	11.54	115.40
	100	101.10	101.10
Corn	1	0.94	93.80
	10	10.34	103.40
	100	101.10	101.10
Fodder	1	1.28	128.00
	10	10.87	108.70
	100	109.70	109.70
Water	1	0.98	98.10
	10	9.73	97.33
	100	99.03	99.033

[46]

3.3 Artificially ripened fruits

Biosensors have also been developed to detect artificially ripened fruits. For commercial purposes, fruits may be artificially ripened [47] which makes the fruits tasteless and unhealthy [48]. In India, most of the climacteric fruits are ripened using industrial-grade calcium carbide [49]. Calcium carbide is composed of phosphorus and arsenic, which are extremely harmful to humans. When calcium carbide reacts with water, it produces a gas called acetylene, which is generally known as carbide gas and is similar to ethylene. Calcium carbide is also a strong and highly reactive gas with carcinogenic properties [50]. Therefore, detection of these artificial ripening agents is of utmost importance. Kathirvelan *et al.* [51] proposed a biosensor that can detect naturally occurring ethylene in fruits. The biosensor was a semiconductor with a titanium dioxide-tungsten trioxide composite material that could detect the release of natural ethylene from fruits. The ethylene molecules would enter the surface of the composite material and fill the voids of the semiconductor, which led to increased conductivity of the material. The amount of ethylene released was detected by the drop in resistance measured by the biosensor. Thus, it can be used to distinguish between artificially and naturally ripened fruits. Maheswaran *et al*. [50] developed a mobile app to determine artificial ripening in mangoes. The proposed system had an efficiency of 91 % in detecting artificially ripened fruits.

4. Biosensors for pesticide residue detection

Pesticides play an indispensable role in agricultural production. However, the presence of pesticide residues in food, water and soil has been linked to serious health problems including cancer, liver damage, reproductive issues and nervous system damage [52]. Determining pesticide residues is therefore essential for ensuring safe food and a healthy environment.

4.1 Herbicide detection

Mirabi-Semnakolaii *et al.* [53] developed a biosensor to detect the herbicide trifluralin in soil. The biosensor consisted of an electrode made up of a composite material containing carbon paste and copper nanowires. Copper nanowires (polycrystalline $Cu(OH)_2$) increased the conductivity of the system. The current developed between the electrodes was measured to identify the presence of trifluralin in the soil sample. The sensitivity of the method was superior to all previously reported methods. The data revealed that the detection limit of the method was about 2.5 times lower than gas chromatography, which was reported to be the most sensitive method. The method was also fast and simple when compared to conventional methods.

Table 4. Trifluralin detected by biosensor in soil samples

Soil samples	Trifluralin added (µg g^{-1})	Trifluralin detected (µg g^{-1})	Recovery (%)
1	7.0	7.11	101.5
2	10.11	10.05	99.3
3	12.01	12.18	101.4

[53]

Atrazine is the most widely used pesticide of the triazine family in crops due to its high efficiency [54,55]. Consumption of atrazine contaminated water causes several health problems such as endocrine and hormone disruption, which may lead to breast cancer and prostate cancer [56,57,58,59]. Supraja *et al.* [60] revealed that a biosensor with zinc oxide nanofibers and anti-atrazine antibodies could be used to detect atrazine. A high-conductivity material was embedded with zinc oxide to increase the conductivity and surface area needed to immobilise antibodies. The anti-atrazine antibody formed a complex with the atrazine molecules. This leads to increase in resistance which was quantified using electrochemical impedance spectroscopy to find out the amount of atrazine in the sample. The proposed biosensor has good stability, selectivity, repeatability, reproducibility and is less prone to interference.

Glyphosate is another important herbicide due to its efficiency in killing weeds and synchronization with the adoption of genetically modified crops that possess glyphosate resistance [61]. Multiple studies have linked chronic glyphosate exposure to various health hazards, such as heart disease [62], non-Hodgkin lymphoma [63], Parkinson's disease [64] and pregnancy issues [65]. Vaghela *et al.* [66] proposed a potentiometric urea biosensor for the detection of glyphosate. In the study, urease enzyme was immobilized on an electrode with gold nanoparticles. Gold nanoparticles enhanced the enzyme activity and the conductivity of the biosensor. During the enzymatic reaction, ammonium ions will be produced, which will be reduced by glyphosate. The amount of glyphosate can be measured by the potential developed between the electrodes. Glyphosate can also be detected using a biosensor that utilises an enzyme called glycine oxidase, which uses glyphosate as a substrate [67]. To measure the amount of glyphosate, the enzyme was immobilized on an electrode, which reacted with the herbicide. The current produced by the system was used to quantify the amount of glyphosate in the samples. The sensor accurately reported glyphosate concentrations in river water, corn residue and soybean residue, with recovery percentages of 92.5 %, 109.1 % and 124.9 %, respectively. The biosensor also showed minimal interference from atrazine, 2,4-D, dicamba, parathion-methyl, paraoxon-methyl, malathion, chlorpyrifos, thiamethoxam, clothianidin and imidacloprid, and the response time was only 150 seconds.

Kim *et al.* [68] reported that 2,4-D could be detected using biosensors with enzymes immobilised on electrodes. An enzyme called tyrosinase was used for the detection of 2,4-D. The activity of tyrosinase on the electrodes was inhibited by 2,4-D, which leads to less potential

between the electrodes. Thus, it was observed that the current decreased after exposure to the pesticide. The results also showed that the biosensor had a low detection limit and enhanced sensitivity of 2,4-D.

Diuron, a substituted phenyl urea herbicide, is used as a broad-spectrum pre-emergent herbicide in a wide variety of crops. The prolonged use of diuron and other phenyl urea herbicides is a big concern since their residues in soil and water exceed the permissible limits [69,70]. Due to its potential toxic and mutagenic effects on plants and animals, it is important to have a detection system that is simple, quick, specific and sensitive to check soil and water contamination. A low-cost electrochemical biosensor for the detection of diuron was developed by Sharma *et al*. [71]. An anti-diuron antibody was utilized in this biosensor and it was immobilised on an electrode to bind with the diuron molecules. The potential developed between the electrodes was used to determine the amount of herbicide in the sample. It was found that the current produced decreased as the concentration of the herbicide increased. The biosensor was sensitive and selective, with low detection limits (1 ppt).

4.2 Insecticide detection

Organophosphorus compounds are widely used in agriculture as insecticides around the world. These neurotoxic compounds irreversibly inhibit the enzyme acetylcholinesterase which is essential for the functioning of the central nervous system in humans and insects [72]. Acetylcholinesterase results in the buildup of the neurotransmitter acetylcholine which interferes with muscular responses. Acetylthiolcholine chloride is usually used as an enzymatic substrate, and the resultant, thiolcholine is either detected electrochemically or by bioassays.

Mishra *et al.* [73] found that a biosensor based on the acetylcholinesterase enzyme can be used to detect three organophosphate insecticides, *viz.,* chlorpyriphos, ethyl paraoxon and malaoxon. The enzyme was immobilised on an electrode. The insecticides inhibited the activity of the enzyme. The amount of pesticide in the sample was detected by measuring the current produced by the apparatus. The accuracy ranged from 90.8 to 98.2 % and the system could be used in milk collection and processing units.

Table 5. Different pesticide quantity detected by biosensor in milk

Pesticide added (ng mL^{-1})	**Pesticide detected (ng mL^{-1})**	**Recovery (%)**
Chlorpyriphos		
5×10^{-11}	4.92×10^{-11}	98.5
5×10^{-7}	4.91×10^{-7}	98.2
Ethyl paraoxon		
5×10^{-9}	4.80×10^{-9}	96.0

5×10^{-7}	4.75×10^{-7}	95.0
Malaoxon		
5×10^{-10}	4.85×10^{-10}	97.0
5×10^{-7}	4.82×10^{-7}	96.5

[73]

Huang *et al.* [74] reported that biosensors could be used to detect organophosphorus pesticides (omethoate) in river water. The biosensor had fluorescent DNA probes that were used to detect the pesticide. The enzyme used in the biosensor was acetylthiocholine, which was hydrolysed to produce thiocholine. Thiocholine is known to react with metal cations like copper. In the presence of the insecticide, the activity of acetylthiocholine was inhibited and copper ions were accumulated in the biosensor. The concentration of insecticides can be determined by detecting the accumulated copper ions (Cu^{2+}) using fluorescence. The copper ions affected the fluorescent property of the biosensor. The fluorescence was found to decrease when pesticide concentrations were increased.

Table 6. Pesticide quantity detected by biosensor in river water

Samples	**Omethoate added (ng mL^{-1})**	**Omethoate detected (ng mL^{-1})**
1	5.00	5.03
2	9.00	9.21
3	20.00	20.14
4	40.00	39.89

[74]

Jin *et al.* [75] developed a portable kit to detect organophosphorus (paraoxon) by using silver ions as metal cations to inhibit the activity of acetylthiocholine. The fluorescence emitted by the biosensor changed from colourless to pale yellow with increasing concentrations of the pesticide. The biosensor had an accuracy of 85.4 to 96.5 %.

Table 7. Pesticide quantity detected by biosensor in pear juice

Pesticide added (ng mL^{-1})	**Pesticide detected (ng mL^{-1})**	**Recovery (%)**
0	-	-
50	42.72	85.4
100	88.67	88.7
500	482.37	96.5

[75]

Zhang *et al*. [76] developed a disposable biosensor to detect organophosphates (paraoxon) and carbamates (carbaryl) in milk. Four types of acetylcholinesterase enzymes were used to make a multienzyme biosensor that could detect trace amounts of pesticides. The

accuracy of the test ranged from 89 to 107 % and pesticide levels of less than 1µgL^{-1} could be detected in milk using this method. Kumar *et al.* [77] developed an optical biosensor with a disposable microbial membrane using *Flavobacterium* sp. to detect methyl parathion. *Flavobacterium* sp. has the organophosphorus hydrolase enzyme, which hydrolyzes methyl parathion into a detectable product, p-nitrophenol. The microbial component was attached to a glass fiber filter which can be disposed of after testing and the reading was taken using the optical biosensor. It was concluded that the apparatus required only small amounts of substrate and could be used to detect methyl parathion with high accuracy. Duford *et al.* [78] reported that enzyme-based biosensors could be used to detect carbofuran in soil and vegetables. The enzyme acetylcholinesterase was used to detect the pesticide. The results revealed that the biosensors were effective and were statistically similar to the conventional method with a low carbofuran detection limit of 0.1 µg g^{-1}.

Anirudhan and Alexander [79] reported that a potentiometric biosensor can be used to detect the organochlorine pesticide lindane. Lindane contains negatively charged chlorine ions, which helps to develop potential between the electrodes. The quantity of the pesticide was noted by detecting the potential developed by the system. The feasibility of the biosensor was tested in Kerala by testing samples of water, fruits and vegetables. It was revealed that the biosensor could selectively detect lindane in these samples with high sensitivity and reproducibility.

4.3 Fungicide detection

The extensive use of fungicides is undesirable due to their negative effects on the environment [80], health risks to farmers [81], the emergence of resistant fungal strains [82,83] and concerns that residues may end up in food products [84]. Choi *et al.* [85] developed an enzyme-based optical biosensor to detect captan in water. The enzyme used was glutathione-S-transferase (GST). The GST enzyme converts two substrates, *viz.,* 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione (GSH), to a yellow product (s-glutathione) that is detected by the optical biosensor [86,87]. Captan acts as an inhibitor in this reaction and stops the production of the yellow product. In the absence of inhibitors, the substrates are completely converted into yellow products, while in the presence of inhibitors, the quantity of yellow products are reduced. The reduction in the yellow product is read by the optical biosensor, which produces a signal to denote the pesticide concentration. The signal produced increased with the concentration of captan.

Chen *et al.* [88] reported that dithiocarbamate fungicide residue can be detected in fruit samples using copper biosensors. Copper nanoparticles were used, which produced orange-reddish fluorescence in their normal state. They reacted with dithiocarbamates to produce a

complex and the fluorescence decreased due to the complex formation. The fluorescence intensity decreased with increased concentrations of the pesticide. The biosensor exhibited selectivity and sensitivity to dithiocarbamates even in the presence of interferences like heavy metals and other fungicides. Koukouvinos *et al.* [89] proposed an optical biosensor for the detection of carbendazim. An anti-carbendazim antibody was used to detect the amount of the fungicide. It was revealed that the biosensor had excellent analytical characteristics and a short analysis time and was ideal for the determination of carbendazim in food and environmental samples. The biosensors developed could be used to detect residues of fungicide and prevent dithiocarbamate contamination.

5. Biosensor for plant disease detection

Biosensors are advanced detection tools in research fields for the detection of airborne pathogens and pesticide residues in foods and beverages [90]. Biosensing techniques have practical applications in the detection of plant pathogens and significant diagnostic results can be achieved through real life applications.

5.1 Fungal diseases

Fusarium is one of the most significant and widespread wilt pathogens causing diseases in crop plants [91]. *Fusarium* sp. can also produce mycotoxin in cereals, fruits, and vegetables [92]. Nozaki [93] reported that *Fusarium* sp. infection in Gerbera could be detected early using ruthenium-red (dye) based biosensors. *Fusarium* sp. produces polygalacturonase enzymes, which caused cell wall degradation in plants. These enzymes can be detected using ruthenium red dye in the biosensor. Multiple polygalacturonase enzymes were tested with varying concentrations, and the biosensor detected the reflected light from those enzymes using a spectral sensor. As the concentration of the enzymes increased, the colour of the solution darkened. Thus, biosensors can be used to detect *Fusarium* sp. infection in plants.

Franco *et al.* [94] proposed a biosensor to detect *Phytophthora palmivora*, a notorious pathogen of cocoa causing black pod rot. DNA hybridization was used in this biosensor, where the DNA of *P. palmivora* was sandwiched between two DNA probes selected for the study. The detection of these hybrids indicated the presence of the pathogen in the samples. The biosensor was also very selective for *Phytophthora* sp., and did not show positive results for other pathogens like *Colletotrichum gloeosporioides*, *Fusarium* sp., and *Lasiodiplodia theobromae*.

Harpaz *et al.* [95] developed a biosensor that could detect the presence of *Colletotrichum gloeosporioides,* which causes anthracnose in fruits. The fungi usually remain quiescent in immature fruit, which cannot be detected visually. They switch to their pathogenic

state only after ripening [96]. Enoyl-CoA-hydratase/isomerase is a marker used to detect the presence of *C. gloeosporioides*. The biosensor used specific DNA that allowed the identification of the marker. The DNA was also modified to produce light signal that denotes the presence of the fungi and the light developed was read by the biosensor. Zamir *et al.* [97] reported that *Colletotrichum gloeosporioides* infection in harvested papaya fruits can also be detected with a biosensor that detects the RNA of the fungi. The biosensor not only allows pathogen detection in fresh agricultural produce, but also identifies the unseen quiescent fungi inside the fruit.

5.2 Viral diseases

Plant virus diseases are extremely dangerous when they occur in staple food crops as they are capable of decreasing food supplies, leading to famines [98,99,100]. In 2014, virus disease pandemics and epidemics were estimated to have a global economic impact of more than $30 billion annually [101]. Therefore, control and detection of these viral diseases are necessary. Berto *et al.* [102] found that biosensors could be used to detect plant viruses such as the Plum Pox Virus (PPV), which wass one of the most devastating viral diseases of stone fruits like peaches, apricots, plums, almonds, cherries, etc. [103]. The virus sensing unit consisted of anti-PPV antibodies, which were placed on gold electrodes. The potential developed between the electrodes determined the presence of the viral pathogen in the sample.

Razmi *et al.* [104] developed a biosensor to detect the presence of tomato yellow leaf curl virus. The biosensor consisted of a DNA probe complementary to the coat protein region of the virus, which was hybridised with the viral DNA. The amount of hybridised DNA was determined by using gold nanoparticles, which changed the colour of the solution in the presence of viral DNA. Shojaei *et al.* [105] detected the presence of citrus tristeza virus using a biosensor. Antibodies against the citrus tristeza virus were used, which formed a complex with the viral particle. The virus infection was determined using fluorescence produced during the complex formation. The developed biosensor could detect samples within a few minutes, and the detection limit was twenty times higher than that of ELISA.

5.3 Bacterial diseases

Bacterial diseases result in severe losses in production, malnutrition and hunger. The responsibility of reducing the impact of these diseases is vested on both the farmers and the government. Indian government spent Rs.1000 crores to combat the bacterial blight of pomegranate during 2003 to 2008, besides loss of Rs. 2318.3 crores by the farmers [106]. Thus, the control of these diseases is of extreme importance. Regiart *et al.* [107] developed an antigen-based biosensor to detect the pathogen *Xanthomonas arboricola*, which caused diseases like brown apical necrosis, blight and canker on apples. The anti-*Xanthomonas arboricola* antibody will bind to the pathogen, and this will produce a current in the system. The

measured current was directly proportional to the level of *Xanthomonas* in the samples. The biosensor diagnosis was three times faster than ELISA and provided significantly higher specificity and sensitivity for the early and *in situ* diagnosis of *Xanthomonas arboricola*.

Tran *et al.* [108] developed a biosensor to detect citrus greening or Huanglongbing (HLB), one of the most devastating bacterial diseases. The plants affected by HLB secrete a protein called SDE1, which was determined using anti-SDE1 antibodies. The protein-antibody complex increased the resistance of the system. Thus, the presence of the bacteria could be determined by the increased resistance of the system. A biosensor for the detection of *Erwinia mallotivora*, which causes papaya dieback was developed by Said *et al.* [109]. A DNA based biosensor with two unique genes specific to the bacteria was utilised for detection. The presence of the genes increased the resistance of the system and the increased resistance can be used to quantify bacteria in the samples. Hidayati and Susilowati [110] proposed a biosensor to confirm the presence of *Clavibacter* sp. in tomato seeds. A specific DNA probe was used to hybridise with the DNA of the bacteria. The detection of these DNA led to the determination of the bacteria.

6. Biosensor for heavy metal detection

Heavy metals, even in trace amounts, cause serious pollution problems and are a threat to the environment and human health due to their non-biodegradable nature [111]. The nonbiodegradable nature of heavy metals results in persistent environmental contamination as they remain in soil and water for extended periods. This can lead to serious health risks, such as cancer and neurological disorders, soil degradation, water pollution, and bioaccumulation in the food chain, impacting both ecosystems and human health. The harmful effects of heavy metals have resulted in regulations to reduce their concentration in nature. Moreover, people are becoming more conscious of the environment, and laws to reduce heavy metal contamination are becoming stricter at both national and international levels [112]. Conventional techniques to analyse heavy metals include cold vapour atomic absorption spectrometry, inductively coupled plasma mass spectrometry, UV visible spectrophotometry and X-ray absorption spectroscopy [113]. Even though these techniques are highly precise, they are expensive, require highly trained personnel, mostly laboratory-bound and are not applicable in the field. There is a need for reliable, efficient and cost-effective technologies to determine the presence of heavy metals [114]. Thus, biosensors can be used for the detection of heavy metals in our environment.

6.1 Heavy metal detection in soil

Liu *et al.* [115] proposed a handheld biosensor to detect mercury content in the soil. Most mercury forms are highly toxic to humans and even low exposure can seriously affect the

central nervous system [116]. The health risks are greater for foetuses and young children than for adults [117]. Mercury transforms to methyl-mercury, which is prone to bioaccumulation in organisms. In this novel biosensor, a protein, *viz.,* MerR protein, was used, which showed activity in the presence of mercury ions. This protein binds with mercury and initiates the synthesis of ethylene in the soil. The ethylene will be released as gas from the soil and quantified using a handheld ethylene sensor. Thus, the estimation of ethylene will provide the quantity of mercury present in the soil. Rathnayake *et al.* [118] found that biosensors could be used to detect heavy metals in soils. A bacterial biosensor was developed using *Bacillus megaterium*, which was sensitive to several heavy metals in soil, such as cadmium, copper and zinc. The bacteria were immobilised in a silica matrix. The bacteria contain a protein called Green fluorescent protein (GFP), which produces fluorescence in the presence of heavy metals, and its intensity decreases with an increase in the concentration of heavy metals. Asif *et al.* [119] also conducted a similar study using the GFP protein from *Escherichia coli* to detect heavy metals such as mercury, lead and zinc.

6.2 Heavy metal detection in irrigation water

Jacob *et al.* [120] reported that biosensors could be used to detect toxic levels of lead in water. Lead ions can easily interfere with important bio molecules in cellular systems, thereby inactivating them and affecting important cellular functions required for normal metabolic activities. The toxicity of lead ions in the human body can result in cardiovascular diseases, neurological, reproductive and developmental disorders and mortality [121]. The biosensor developed for this study used *Aspergillus* sp., which can produce fluorescence through the production of ZnS. The study found that fluorescence intensity decreased with the increase in lead ions and the fluorescence was measured using a spectrophotometer.

Naik and Jujjavarapu [122] developed a self-powered and reusable single chambered cylindrical microbial fuel cell for toxicity detection in water. Microbial fuel cell is an electrochemical device that converts chemical energy in to electrical energy by microbial metabolic pathways. This apparatus was used to detect heavy metals such as copper, chromium, zinc and nickel. These heavy metals were used as a solution and injected into the biosensor. In the presence of the heavy metals, a voltage drop was noted. This drop in voltage provided information on the presence and quantity of heavy metals present in the water sample.

6.3 Heavy metal detection in plants

Plants are constantly exposed to heavy metal pollution, which could be detrimental to plants and the ecosystem [123,124]. The adverse impact of heavy metal stress on plants begins with invisible damage to the plants, and then the damage increases after long-term exposure. Heavy metal stress decreases the content of chlorophyll in plant leaves before it causes visible

damage to plants [125,126]. Therefore, early heavy metal detection is extremely important. Wang *et al.* [127] developed a biosensor that utilizes a defensive plant protein called vitronectin-like protein that resists exogenous harmful factors to serve as a biomarker for detecting the response of plants to cadmium and lead. An antibody called an anti-vitronectin-like protein antibody was used to form a complex with the proteins produced, and an electrochemical biosensor was used to detect the complexes formed. It was found that the vitronectin-like protein content increased on the surface of plant leaf cells with increasing content of cadmium and lead. The resistance of the system increased with increased concentration of heavy metals. Thus, the biosensor could be used to identify invisible damage that occurs to plants due to heavy metal stress. Zhang *et al.* [121] found that lead could be detected in leafy vegetables using DNAzymes. A DNAzyme selective for lead was designed and used for detection. The biosensor was able to detect lead in trace amounts (ppt), and could be used to find the accumulation of lead in leafy vegetables.

7. Conclusion

Application of biosensors in the monitoring of safe food has been a research focus for decades due to their characteristics such as simplicity, sensitivity and low cost [128]. The use of biosensors in agriculture is mainly for the detection of pesticide residues, heavy metal contamination in plants, soil and water, pathogens in crops and ensuring good-quality food. Biosensors will play a key role in sustainable agriculture as they help to minimise resource usage and adapt strategies that support best agricultural practices [129]. For example, continuous monitoring of plant health and the adequate detection of plant pathogens will minimise the amount of fertiliser and pesticide used [130]. This not only reduces food contamination but also saves environmental resources. The Internet of Things (IoT) will be a key part of increasing food safety in the future [131], as it provides the possibility to store and share large data sets. For the traceability of food, the use of block chain technology is also a promising approach [132]. In the upcoming years, the integration of biosensors with these technologies will contribute to the advancement of food safety and sustainable agriculture.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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