NANO-ENABLED BIOSENSORS FOR PEST AND DISEASE DETECTION

CHAPTER 4

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ABSTRACT: This chapter explores the fundamental principles, design strategies, and applications of nano-enabled biosensors in agricultural diagnostics. Nanomaterials such as gold nanoparticles, quantum dots, carbon nanotubes, graphene, and magnetic nanoparticles have been extensively utilized to enhance biosensor performance through improved signal transduction, increased surface area for biomolecule immobilization, and unique optical and electrochemical properties. The chapter examines various biosensor configurations including electrochemical, optical, piezoelectric, and field-effect transistor-based platforms, highlighting their mechanisms of action and comparative advantages. Specific applications in detecting plant pathogens (bacteria, viruses, fungi), insect pests, and phytotoxins are discussed with emphasis on real-world implementation challenges and success stories. The integration of nano-biosensors with emerging technologies such as smartphone-based detection systems, Internet of Things (IoT) platforms, and artificial intelligence for data analysis is presented as a pathway toward precision agriculture. Critical considerations including biocompatibility, environmental stability, cost-effectiveness, and regulatory frameworks are addressed. The chapter concludes with future perspectives on next-generation nano-biosensors, including multiplexed detection systems, wearable sensors for continuous monitoring, and fully integrated lab-on-chip devices that promise to transform pest and disease management strategies in sustainable agriculture.

Keywords: Biosensors in agricultural diagnostics, gold nanoparticles, quantum dots, carbon nanotubes, graphene, and magnetic nanoparticles, nano-biosensors, multiplexed detection systems, wearable sensors

INTRODUCTION

Agriculture faces increasing pressure to enhance productivity while reducing environmental impacts, a challenge further intensified by pests and diseases that cause annual crop losses exceeding 20–40% worldwide (Savary *et al.*, 2019). Conventional approaches for plant pathogen and pest detection—including visual diagnosis, culture-based assays, and polymerase chain reaction (PCR) techniques—remain widely used; however, they are constrained by long processing times, dependence on specialized laboratory infrastructure, and limited capacity for real-time monitoring (Fang & Ramasamy 2015). These limitations often delay timely intervention, increasing crop vulnerability to disease outbreaks. Traditional disease management strategies, such as field screening of crop germplasm and chemical control, continue to play a critical role in resistance breeding and crop protection (Makwana *et al.*, 2023; Kharte *et al.*, 2023; Kulkarni & Sabeena 2023).

The emergence of nanotechnology has initiated a paradigm shift in agricultural diagnostics by enabling the development of highly sensitive, portable, and cost-effective biosensing platforms capable of detecting pathogens and pests at extremely low concentrations. Nano-enabled biosensors integrate nanotechnology, biotechnology, and sensor engineering to exploit the unique physicochemical properties of nanomaterials, thereby enhancing detection efficiency and accuracy (Vikesland, 2018). These properties include high surface-to-volume ratios, quantum confinement effects, enhanced catalytic activity, and distinctive optical and electronic characteristics. When coupled with biological recognition elements such as antibodies, enzymes, nucleic acids, or aptamers, nanomaterials form highly selective biosensing platforms capable of detecting analytes at femtomolar levels (Kumar *et al.*, 2019). Recent agri-bio innovations emphasize the growing importance of such advanced diagnostic tools in modern farming systems (Lal *et al.*, 2024).

The miniaturization enabled by nanotechnology has facilitated the development of portable, point-of-care diagnostic devices suitable for on-site agricultural applications, where rapid decision-making is essential. Early and accurate detection allows farmers to implement timely control measures, complementing integrated crop management strategies and reducing dependence on excessive chemical inputs. These advancements align with sustainable agriculture objectives that emphasize efficient resource utilization and minimal environmental disturbance (Lal *et al.*, 2024).

Beyond pathogen detection, nano-biosensors support precision agriculture by enabling early intervention, real-time pest population monitoring, and targeted application of pesticides and treatments, thereby reducing chemical overuse and environmental contamination (Fraceto *et al.*, 2016). The ability to identify pathogens prior to the appearance of visible symptoms transforms pest and disease management from reactive control to proactive prevention. Multiplexed nano-biosensor platforms further enhance diagnostic efficiency by allowing simultaneous detection of multiple pathogens or stress indicators from a single sample. Such innovations complement broader agricultural management practices, including integrated nutrient management and optimized irrigation strategies, which collectively enhance crop health and productivity (Kumar *et al*., 2024).

In addition, advances in nanomaterial synthesis, including the development of biologically derived nanoparticles, have expanded the scope of nano-enabled agricultural applications. For example, herbal nanoparticle formulations demonstrate the potential of green nanotechnology approaches that reduce ecological risks while offering functional benefits (Kumari *et al*., 2023). Together, nano-enabled biosensing technologies, resistant cultivar development, and integrated management practices represent a holistic approach to sustainable crop protection. This chapter therefore examines the principles, fabrication strategies, performance characteristics, and practical applications of nano-enabled biosensors in agriculture, with a focus on their role in detecting biotic stresses and supporting resilient, sustainable farming systems.

Fundamental Principles of Nano-enabled Biosensors

Biosensors are analytical devices that combine a biological recognition element with a physicochemical transducer to detect specific analytes. The integration of nanomaterials into biosensor architecture fundamentally enhances each component of this system. The biological recognition element, which may consist of antibodies, enzymes, nucleic acid probes, bacteriophages, or aptamers, provides specificity for the target analyte through complementary binding interactions (Barhoum *et al.*, 2019). Nanomaterials serve multiple functions in this context: they provide high-surface-area substrates for immobilizing recognition elements at high densities, they can act as signal amplification agents through their intrinsic optical or electrochemical properties, and they may function as transduction elements themselves, directly converting biological recognition events into measurable signals.

The transduction mechanisms employed in nano-biosensors span several physical domains. Electrochemical transduction, one of the most widely implemented approaches, relies on measuring changes in electrical current, potential, impedance, or conductance resulting from biorecognition events (Pumera *et al.*, 2007). Nanomaterials such as carbon nanotubes, graphene, and metallic nanoparticles significantly enhance electrochemical signals through their excellent electrical conductivity, high electrocatalytic activity, and ability to facilitate electron transfer between biological redox centers and electrode surfaces. Optical transduction methods, including fluorescence, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), and colorimetric detection, benefit from the unique optical properties of quantum dots, gold nanoparticles, and plasmonic nanostructures (Alvarez-Puebla & Liz-Marzán, 2010). These nanomaterials exhibit size-dependent optical properties, high extinction coefficients, and the ability to generate enhanced electromagnetic fields that amplify spectroscopic signals by orders of magnitude.

Piezoelectric transduction, employed in quartz crystal microbalance (QCM) and surface acoustic wave (SAW) devices, detects mass changes on sensor surfaces resulting from analyte binding (Pohanka, 2018). The incorporation of nanostructured surfaces increases the effective binding area and enhances sensitivity to minute mass changes. Field-effect transistor (FET)-based biosensors, particularly those utilizing carbon nanotubes or graphene as the semiconductor channel, detect changes in surface charge or potential upon analyte binding, offering label-free, real-time detection with exceptional sensitivity (Kaisti, 2017). The performance metrics of nano-biosensors—sensitivity, selectivity, limit of detection, response time, and dynamic range—are all significantly improved through judicious selection and integration of nanomaterials optimized for specific detection challenges.

Nanomaterials for Biosensor Applications

The palette of nanomaterials available for biosensor construction is remarkably diverse, each offering distinct advantages for specific applications. Gold nanoparticles (AuNPs) represent one of the most extensively studied nanomaterials in biosensing, valued for their excellent biocompatibility, ease of functionalization through thiol chemistry, tunable optical properties, and stability (Saha *et al*., 2012). AuNPs exhibit strong surface plasmon resonance absorption in the visible spectrum, with absorption wavelength dependent on particle size, shape, and aggregation state. This property has been exploited in colorimetric biosensors where analyte-induced aggregation or disaggregation of AuNPs produces visible color changes detectable by the naked eye or simple spectrophotometers. The high surface area of AuNPs also enables dense loading of recognition elements, while their excellent conductivity enhances electrochemical sensor performance.

Carbon-based nanomaterials, including carbon nanotubes (CNTs), graphene, and carbon quantum dots, have emerged as transformative materials in biosensor development (Maduraiveeran *et al*., 2018). Single-walled and multi-walled carbon nanotubes possess exceptional electrical conductivity, high mechanical strength, and large surface areas, making them ideal electrode materials for electrochemical biosensors. The π-electron-rich structure of CNTs facilitates strong π-π stacking interactions with aromatic molecules and enables non-covalent functionalization strategies that preserve the electronic properties of the nanotubes. Graphene, a two-dimensional sheet of sp2-bonded carbon atoms, offers even larger surface areas, ambipolar electrical transport, and transparency, attributes that have been leveraged in FET-based biosensors, transparent electrodes, and optical sensing platforms. Carbon quantum dots, with their size-dependent fluorescence, low toxicity, and photostability, provide excellent alternatives to semiconductor quantum dots for fluorescence-based detection.

Semiconductor quantum dots (QDs), typically composed of cadmium selenide, cadmium telluride, or more recently, indium phosphide, exhibit size-tunable fluorescence emission, high quantum yields, broad absorption spectra, narrow emission peaks, and resistance to photobleaching (Algar *et al*., 2011). These properties make QDs superior to organic fluorophores for multiplexed detection, as multiple QD populations with different emission wavelengths can be simultaneously excited by a single light source. However, concerns about heavy metal toxicity have driven development of cadmium-free alternatives and core-shell structures with enhanced biocompatibility. Magnetic nanoparticles, particularly iron oxide nanoparticles, offer unique advantages in biosensing through their responsiveness to external magnetic fields, enabling magnetic separation and concentration of target analytes from complex matrices, thereby improving detection limits and reducing interference (Gao *et al*., 2007).

Metal oxide nanoparticles including titanium dioxide, zinc oxide, and cerium oxide exhibit excellent chemical stability, biocompatibility, and catalytic properties useful in both electrochemical and optical biosensors (Ansari *et al*., 2013). Zinc oxide nanostructures with various morphologies (nanowires, nanorods, nanoflowers) provide high isoelectric points that favor immobilization of negatively charged biomolecules through electrostatic interactions. Upconversion nanoparticles, containing lanthanide ions, convert near-infrared excitation to visible or ultraviolet emission, enabling detection in biological samples with minimal background interference from autofluorescence. The selection of nanomaterial for a specific biosensor application depends on multiple factors including the target analyte, required sensitivity, detection method, cost constraints, and environmental considerations, with many modern biosensors employing hybrid nanocomposites that combine complementary properties of different nanomaterials to achieve optimal performance.

Table 1: Types of Nanomaterials Used in Biosensors for Agricultural Applications.

Nanomaterial Type	**Key Properties**	**Biosensor Application**	**Detection Method**	**Representative Examples**	**References**
Gold nanoparticles (AuNPs)	Surface plasmon resonance, high surface area, easy functionalization, excellent biocompatibility	Colorimetric detection, LSPR sensing, signal amplification in electrochemical sensors	Colorimetric, optical, electrochemical	Detection of Phytophthora infestans, Xanthomonas species, various plant viruses	Aldewachi *et al*., 2018; Kashyap *et al*., 2022
Carbon nanotubes (CNTs)	High electrical conductivity, large surface area, strong mechanical properties	Electrode materials for electrochemical sensors, field-effect transistors	Electrochemical (amperometric, impedimetric)	Ralstonia solanacearum detection, aphid salivary protein sensing	Pumera *et al*., 2007; Kumar *et al*., 2019
Graphene and graphene oxide	Exceptional conductivity, large surface area, transparency, mechanical strength	FET-based sensors, electrochemical transducers, transparent electrodes	Electrochemical, optical, FET-based	Bacterial pathogen detection, virus sensing, multi-pathogen platforms	Kaisti, 2017; Maduraiveeran *et al*., 2018
Quantum dots (QDs)	Size-tunable fluorescence, high quantum yield, photostability, broad absorption	Fluorescence-based detection, FRET sensors, multiplexed detection	Fluorescence, FRET	Cucumber mosaic virus, multiplexed bacterial detection, DNA hybridization assays	Algar *et al*., 2011; Resch-Genger *et al*., 2008
Magnetic nanoparticles	Magnetic responsiveness, enables separation and concentration	Pre-concentration of analytes, magnetic immunoassays	Electrochemical, optical (after separation)	Aphid protein detection, pathogen capture from complex matrices	Gao *et al*., 2007; Kumar *et al*., 2019
Silver nanoparticles	Strong SERS enhancement, antimicrobial properties, plasmon resonance	SERS substrates, colorimetric detection	SERS, colorimetric	Fungal species discrimination, bacterial pathogen identification	Pilot *et al*., 2019
Metal oxide nanoparticles (ZnO, TiO_2)	Chemical stability, biocompatibility, catalytic activity, varied morphologies	Electrochemical sensors, photocatalytic detection	Electrochemical, optical	Ergosterol detection (fungal quantification),	Ansari *et al*., 2013; Bahadir & Sezgintürk, 2016

				virus immunosensors	
Carbon quantum dots	Fluorescence, low toxicity, photostability, easy functionalization	Fluorescent aptasensors, imaging applications	Fluorescence	Fusarium graminearum detection, stress hormone monitoring	Luo *et al*., 2020

Table 2: Performance Characteristics of Selected Nano-biosensors for Agricultural Pathogen Detection.

Target Pathogen/Analyte	Biosensor Type	Nanomaterial Platform	Recognition Element	Detection Limit	Response Time	Sample Matrix	Reference
Xanthomonas axonopodis pv. punicae	Electrochemical impedimetric	Graphene oxide/AuNPs composite	Monoclonal antibodies	10^2 CFU/mL	60 min	Plant extract	Fang & Ramasamy, 2015
Ralstonia solanacearum	Electrochemical amperometric	Multi-walled CNTs	Polyclonal antibodies	10^3 CFU/mL	45 min	Soil suspension	Kumar *et al*., 2019
Potato virus Y (PVY)	Electrochemical impedimetric	AuNPs/polyaniline/ TiO_2	Monoclonal antibodies	0.1 ng/mL	30 min	Leaf extract	Bahadir & Sezgintürk, 2016
Tobacco mosaic virus (TMV)	Electrochemical voltammetric	Graphene quantum dots	Aptamers	0.5 fM	40 min	Buffer/plant sap	Luo *et al*., 2020
Fusarium oxysporum	Electrochemical DPV	Gold nanoparticles	DNA probes	1.2 pM (target DNA)	90 min	DNA extract	Vikesland, 2018
Phytophthora infestans	Colorimetric	Gold nanoparticles	Polyclonal antibodies	10^4 spores/ mL	30 min	Spore suspension	Kashyap *et al*., 2022
Cucumber mosaic virus	SERS immunoassay	AuNPs with Raman reporters	Monoclonal antibodies	0.5 ng/mL	20 min	Crude plant extract	Pilot *et al*., 2019
Fusarium graminearum	Fluorescence	Carbon quantum dots	Aptamers	10 spores/ mL	35 min	Buffer/spore suspension	Luo *et al*., 2020
Candidatus Liberibacter asiaticus	Electrochemical with LAMP	CNT-modified electrodes	DNA primers	10 cells/mL	120 min	Leaf extract	Duan *et al*., 2009
Deoxynivalenol (mycotoxin)	Immunochromatographic	Gold nanoparticles	Antibodies	0.5 mg/kg	10 min	Grain extract	Zhang *et al*., 2020
Codlemone (pheromone)	Electrochemical voltammetric	AuNPs/molecular imprinted polymer	Synthetic receptors	ppt range	15 min	Air sample	Abdullah *et al*., 2020
Jasmonic acid (stress hormone)	Fluorescence	Quantum dots	Enzyme-based	10 nM	Real-time	In planta	Zhang *et al*., 2020

Table 3: Advantages and Limitations of Different Nano-biosensor Platforms for Agricultural Applications

Biosensor Platform	Key Advantages	Primary Limitations	Best Applications	Technology Readiness	Cost Considerations
Electrochemical (amperometric/voltammetric)	High sensitivity, rapid response, miniaturizable, low power, quantitative results	Requires electrodes and potentiostat, susceptible to fouling, interference from electroactive species	Laboratory and field detection of pathogens, continuous monitoring systems	High (commercial devices available)	Moderate (electrode and electronics costs)
Electrochemical (impedimetric)	Label-free detection, high sensitivity, real-	Complex data interpretation, affected by non-specific binding,	Real-time pathogen detection, monitoring	High (commercial devices available)	Moderate to high (electronics costs)

	time monitoring, simple principle	requires stable reference electrode	binding kinetics, FET-based sensors		
Colorimetric	Visual readout (no instruments needed), simple, low cost, suitable for resource-limited settings	Semi-quantitative, lower sensitivity than other methods, subjective interpretation	Field screening tests, rapid diagnostics for smallholder farmers, POC testing	Very high (commercial products available)	Low (minimal equipment required)
Fluorescence	High sensitivity, multiplexing capability, well-established, compatible with microscopy	Requires excitation source and detector, photobleaching of some fluorophores, autofluorescence interference	Multiplexed pathogen detection, laboratory diagnostics, imaging applications	High (established technology)	Moderate (light source and detector costs)
SERS	Extremely high sensitivity, molecular fingerprinting, multiplexing potential, label-free option	Requires specialized substrates, reproducibility challenges, expensive instrumentation	Strain differentiation, molecular identification, high-sensitivity detection	Medium (mostly research-stage)	High (Raman spectrometer costs)
LSPR/SPR	Label-free, real-time monitoring, high sensitivity, no sample labeling needed	Sensitive to temperature and refractive index changes, complex optics, surface fouling	Real-time binding studies, screening applications, sensor networks	Medium to high	High (optical components)
FET-based	Ultrahigh sensitivity, label-free, miniaturizable, potential for integration	Complex fabrication, sensitive to environmental conditions, ionic strength effects	Ultrasensitive detection, continuous monitoring, integrated sensor arrays	Medium (emerging commercial products)	High (fabrication complexity)
Piezoelectric (QCM/SAW)	Label-free, measures mass directly, real-time, reusable sensors	Lower sensitivity than electrochemical methods, affected by viscosity and temperature	Monitoring biomolecular interactions, air-borne analyte detection	Medium to high	Moderate to high (crystal and oscillator costs)
Paper-based microfluidic	Extremely low cost, disposable, no power required, simple operation, suitable for developing regions	Lower sensitivity, qualitative/semi-quantitative, single-use, limited multiplexing	Rapid field diagnostics, resource-limited settings, extension services	High (commercial products exist)	Very low (paper substrate costs)

Electrochemical Nano-biosensors for Pathogen Detection

Electrochemical bsiosensors have gained prominence in agricultural diagnostics due to their high sensitivity, rapid response, simple instrumentation, and amenability to miniaturization (Cesewski & Johnson 2020). These sensors measure electrical signals amperometric (current), potentiometric (potential), or impedimetric (impedance) generated by electrochemical reactions involving the target analyte or resulting from changes in electrode surface properties upon analyte binding. The incorporation of nanomaterials significantly enhances electrochemical biosensor performance through multiple mechanisms: increased electroactive surface area, improved electron transfers kinetics, electrocatalytic activity that reduces overpotentials, and provision of high-density sites for biomolecule immobilization.

Nanomaterial-modified electrodes have demonstrated remarkable success in detecting plant pathogenic bacteria. For instance, graphene oxide/gold nanoparticle nanocomposites have been employed to develop immunosensors for detecting Xanthomonas axonopodis pv. punicae, the causative agent of bacterial blight in pomegranate, achieving detection limits in the range of 10^2 CFU/mL within 60 minutes (Fang & Ramasamy 2015). The nanocomposite structure provides both high conductivity and abundant sites for antibody immobilization, while the electrochemical impedance spectroscopy technique employed offers label-free detection. Similarly, carbon nanotube-based electrochemical sensors have been developed for detecting Ralstonia solanacearum, a devastating bacterial pathogen affecting numerous crops including potato, tomato, and banana. Multi-walled carbon nanotubes functionalized with specific antibodies demonstrated detection capabilities down to 10^3 CFU/mL, with excellent selectivity even in the presence of other bacterial species (Kumar *et al.*, 2019).

Viral pathogen detection presents particular challenges due to the small size of viruses and often low concentrations in plant tissues during early infection stages. Nano-enabled electrochemical biosensors have addressed these challenges through innovative design strategies. An impedimetric biosensor utilizing gold nanoparticles decorated on a polyaniline/titanium dioxide nanocomposite successfully detected Potato virus Y (PVY) at concentrations as low as 0.1 ng/mL (Bahadir & Sezgintürk 2016). The sensor employed monoclonal antibodies specific to PVY coat protein, with binding events detected through changes in charge transfer resistance. Aptamer-based electrochemical sensors have also shown promise, offering advantages over antibody-based sensors in terms of stability, cost, and ease of production. Graphene quantum dot-enhanced electrochemical aptasensors for Tobacco mosaic virus (TMV) achieved femtomolar detection limits, demonstrating the potential of aptamer-nanomaterial combinations in viral diagnostics.

Fungal pathogen detection through electrochemical nano-biosensors has focused primarily on detecting specific metabolites, cell wall components, or DNA sequences characteristic of pathogenic fungi. An amperometric biosensor based on platinum nanoparticles embedded in a chitosan matrix was developed for detecting ergosterol, a sterol specific to fungal cell membranes, enabling indirect quantification of fungal biomass in infected plant tissues (Vikesland, 2018). DNA-based electrochemical sensors utilizing gold nanoparticle signal amplification have successfully detected Fusarium oxysporum, a widespread soilborne pathogen, through hybridization with species-specific oligonucleotide probes. The sensor achieved detection limits of 1.2×10^{-12} M of target DNA, corresponding to early-stage infection levels. More recently, CRISPR-Cas systems have been integrated with electrochemical nano-biosensors, leveraging the sequence-specific recognition capabilities of CRISPR-Cas proteins combined with the signal amplification provided by nanomaterials to achieve unprecedented sensitivity and specificity in detecting fungal pathogen DNA.

Optical Nano-biosensors for Agricultural Diagnostics

Optical detection methods offer several advantages for biosensing applications, including the potential for label-free detection, non-destructive measurement, remote sensing capabilities, and compatibility with multiplex detection formats (Luo *et al.*, 2020). Nano-enabled optical biosensors exploit the unique light-matter interactions that occur at the nanoscale, including surface plasmon resonance, quantum confinement effects, and electromagnetic field enhancement. These phenomena enable detection sensitivities approaching single-molecule levels while maintaining specificity through appropriate biorecognition elements.

Colorimetric biosensors represent the simplest category of optical nano-biosensors, typically employing gold or silver nanoparticles whose aggregation state changes in response to target analyte presence. The color change resulting from aggregation or disaggregation can be observed visually or quantified using basic spectrophotometry, making these sensors particularly attractive for resource-limited settings. A colorimetric sensor for detecting Phytophthora infestans, the causal agent of late blight in potato and tomato, utilized antibody-functionalized gold nanoparticles that aggregated in the presence of pathogen-specific surface proteins, producing a visible color shift from red to purple (Kashyap *et al*., 2022). The sensor demonstrated detection limits of 10^4 spores/mL and provided results within 30 minutes, significantly faster than traditional culture-based methods requiring several days.

Surface-enhanced Raman spectroscopy (SERS) biosensors leverage the enormous electromagnetic field enhancement that occurs near the surface of plasmonic nanostructures, particularly gold and silver nanoparticles with sharp features or nanoscale gaps (Pilot *et al*., 2019). This enhancement can amplify Raman signals by factors of 10^6 to 10^{10}, enabling detection of molecular vibrations from minute quantities of analyte. SERS-based biosensors have been developed for detecting various plant pathogens through their unique molecular fingerprints. A SERS immunoassay for detecting Cucumber mosaic virus employed gold nanoparticles conjugated with antibodies and Raman reporter molecules, achieving detection limits of 0.5 ng/mL in crude plant extracts. The technique's ability to distinguish between closely related viral strains through subtle differences in spectral signatures provides valuable epidemiological information. SERS sensors utilizing silver nanorod arrays have also demonstrated rapid discrimination between different fungal species based on their cell wall compositions, with potential applications in identifying pathogenic versus beneficial fungal species in agricultural soils.

Fluorescence-based nano-biosensors exploit the superior photophysical properties of quantum dots and fluorescent nanoparticles compared to conventional organic dyes (Resch-Genger *et al.*, 2008). Quantum dot-based fluorescence resonance energy transfer (FRET) sensors have been developed for detecting plant pathogen DNA sequences, with target hybridization bringing donor and acceptor QDs into proximity, enabling energy transfer and altered emission profiles. A multiplexed fluorescence sensor utilizing different-colored quantum dots enabled simultaneous detection of three bacterial pathogens Pseudomonas syringae, Erwinia amylovora, and Xanthomonas campestris in a single assay, demonstrating the power of nanomaterial-based multiplexing for comprehensive disease diagnosis. Carbon quantum dots have emerged as promising alternatives to semiconductor QDs, offering comparable fluorescence properties with reduced toxicity concerns. A carbon quantum dot-based fluorescent aptasensor for detecting Fusarium graminearum achieved detection limits of 10 spores/mL, with the aptamer providing high specificity and the carbon QDs offering stable, pH-independent fluorescence suitable for diverse agricultural sample matrices.

Localized surface plasmon resonance (LSPR) sensors based on gold or silver nanoparticles fixed on surfaces detect changes in local refractive index resulting from biomolecular binding events, manifested as shifts in the plasmon resonance wavelength (Masson *et al.*, 2017). Unlike conventional SPR sensors requiring bulky prism-coupling arrangements, LSPR sensors can be miniaturized and adapted to various formats including fiber optics and microfluidic chips. An LSPR biosensor for detecting plant viruses utilized gold nanorods functionalized with virus-specific antibodies, with virus binding causing measurable shifts in the longitudinal plasmon resonance peak. The sensor demonstrated rapid detection (less than 15 minutes) and high sensitivity (femtomolar range) without requiring sample amplification or labeling steps. The development of smartphone-based LSPR readers has further enhanced the accessibility of this technology for field applications, with several prototypes demonstrating successful pathogen detection in agricultural settings using only a smartphone camera and simple optical attachments.

Pest Detection Using Nano-biosensors

While much of the nano-biosensor research in agriculture has focused on pathogen detection, the detection of insect pests and the damage they cause represents another critical application area. Direct detection of insects in field conditions remains challenging, but nano-biosensors have proven effective in detecting pest-specific biomolecules, including pheromones, salivary proteins, and metabolites associated with pest feeding damage (Singh *et al.*, 2021). These approaches enable early detection of pest presence before visible damage occurs, allowing for timely intervention and reduced pesticide use.

Electrochemical biosensors have been developed for detecting pheromones, the volatile organic compounds insects use for chemical communication. An electrochemical sensor utilizing gold nanoparticles and molecularly imprinted polymers (MIPs) was designed to detect codling moth pheromone (codlemone), a key target for monitoring this devastating apple pest (Abdullah *et al.*, 2020). The MIP provided synthetic recognition sites complementary to the pheromone structure, while gold nanoparticles enhanced the electrochemical signal. The sensor achieved detection limits in the parts-per-trillion range, comparable to insect olfactory sensitivity, enabling detection of pest presence at very low population densities. Similar approaches have been applied to detecting pheromones of other major pests including Mediterranean fruit fly, cotton bollworm, and stored grain pests.

Detection of insect-derived proteins and enzymes in plant tissues offers another avenue for pest detection. Many piercing-sucking insects inject saliva containing specific proteins into plant tissues during feeding. An immunosensor based on magnetic nanoparticles conjugated with antibodies against aphid salivary proteins was developed to detect aphid feeding on wheat plants (Kumar *et al.*, 2019). The magnetic nanoparticles enabled efficient capture and concentration of target proteins from plant extracts, while subsequent electrochemical detection on carbon nanotube-modified electrodes provided high sensitivity. The sensor could detect aphid feeding within 6 hours of infestation, well before visible damage symptoms appeared, demonstrating the potential for ultra-early pest detection.

Nano-biosensors have also been employed to detect plant stress metabolites that accumulate in response to pest feeding damage. When insects feed on plants, the damaged tissues produce elevated levels of stress hormones such as jasmonic acid and salicylic acid, along with volatile organic compounds (VOCs) that serve as distress signals. A fluorescence-based nanosensor utilizing quantum dots conjugated with enzymes sensitive to jasmonic acid was developed to monitor plant stress responses in real-time (Giraldo *et al.*, 2019). The sensor could be infiltrated into plant leaves, where it provided continuous monitoring of stress hormone levels through changes in fluorescence intensity. This approach enables not only pest detection but also assessment of the severity of pest damage and the effectiveness of pest management interventions. More recently, electronic nose systems incorporating arrays of nanomaterial-based gas sensors have demonstrated the ability to detect and discriminate between VOC profiles associated with different types of pest damage, providing a non-invasive method for identifying the specific pest attacking a crop.

Integration with Smart Agriculture Platforms

The true transformative potential of nano-biosensors in agriculture emerges through their integration with digital technologies, creating comprehensive smart agriculture systems that enable data-driven decision-making (Farooq *et al*., 2020). The miniaturization and low power consumption enabled by nanotechnology allow nano-biosensors to be deployed as nodes in wireless sensor networks distributed throughout agricultural fields. These sensor networks, connected via Internet of Things (IoT) platforms, can provide continuous, real-time monitoring of pest and disease pressure across entire farms, enabling precision targeting of interventions only where and when needed.

Smartphone-based biosensor readout systems represent a particularly impactful integration strategy, leveraging the ubiquity of smartphones to provide sophisticated analytical capabilities to farmers without requiring dedicated laboratory equipment (Roda *et al*., 2014). Several research groups have developed smartphone attachments that enable colorimetric, fluorescence, or chemiluminescence detection of nano-biosensor signals, with custom applications providing user-friendly interfaces for data collection and interpretation. A smartphone-based system for detecting plant viruses utilized a microfluidic paper device incorporating gold nanoparticle-based colorimetric detection, with the smartphone camera capturing color changes and a custom app providing semi-quantitative analysis and data uploading to cloud servers for epidemiological tracking. Field trials demonstrated high concordance between smartphone-based measurements and laboratory spectrophotometer readings, validating the approach for practical agricultural use.

Artificial intelligence and machine learning algorithms are increasingly being integrated with nano-biosensor systems to enhance their analytical capabilities and predictive power (Liakos *et al*., 2018). Machine learning models can be trained to interpret complex sensor signals, discriminate between closely related pathogens, compensate for environmental factors affecting sensor performance, and predict disease progression based on current sensor readings combined with weather data and crop growth models. A deep learning system integrated with SERS nano-biosensor data demonstrated 97% accuracy in classifying six different plant pathogenic bacteria, outperforming traditional chemometric analysis methods. Furthermore, the integration of biosensor data with agricultural decision support systems enables automated triggering of responses such as targeted pesticide application through precision spraying systems or activation of environmental controls in greenhouse settings.

The development of self-powered nano-biosensors represents an important advance toward fully autonomous agricultural monitoring systems. These sensors harvest energy from their environment through solar cells, thermoelectric generators, or even bio-fuel cells powered by plant or soil metabolites eliminating the need for battery replacement in distributed sensor networks (Jiang *et al*., 2020). A self-powered biosensor for detecting soil-borne pathogens utilized a microbial fuel cell integrated with a potentiometric nano-biosensor, with the current generated by soil bacteria providing both power and a reference signal for pathogen detection. Such innovations move closer to the vision of maintenance-free, long-term deployment of biosensor networks throughout agricultural landscapes, providing continuous vigilance against pest and disease threats.

CHALLENGES AND LIMITATIONS

Despite the remarkable advances in nano-biosensor technology and numerous proof-of-concept demonstrations, several significant challenges must be addressed before these technologies can achieve widespread commercial adoption in agriculture (Wolfbeis, 2015). The transition from controlled laboratory conditions to the complex, variable environment of agricultural fields presents particular difficulties. Agricultural samples plant tissues, soil, irrigation water contain numerous interfering substances including proteins, polysaccharides, phenolic compounds, and humic acids that can affect sensor performance through non-specific binding, fouling of sensor surfaces, or interference with detection chemistry. Many nano-biosensors developed and validated in buffer solutions show degraded performance when applied to real agricultural matrices, necessitating extensive sample preparation that diminishes the advantages of rapid, on-site detection.

Environmental stability represents another critical challenge, as biosensors deployed in agricultural settings must function reliably across wide ranges of temperature, humidity, pH, and exposure to ultraviolet radiation. Biological recognition elements, particularly antibodies and enzymes, can denature under field conditions, while nanomaterial properties may change due to aggregation, oxidation, or dissolution. Encapsulation strategies and protective coatings can enhance stability but may also reduce sensor sensitivity or increase response times (Hussain *et al*., 2021). The development of biosensors utilizing more stable recognition elements such as aptamers, peptides, or molecularly imprinted polymers addresses some of these concerns, though often at the cost of reduced specificity or binding affinity compared to antibodies.

Reproducibility and quality control in nano-biosensor fabrication present ongoing challenges, as many published biosensor designs rely on manual assembly processes that yield significant device-to-device variability. The properties of nanomaterials themselves size distribution, shape, surface chemistry, crystallinity can vary between synthesis batches, affecting sensor performance. Industrial-scale manufacturing of nano-biosensors requires robust, automated fabrication processes with rigorous quality control, capabilities currently lacking for many advanced biosensor designs (Hanafi *et al*., 2020). The cost of nano-biosensors, while potentially lower than traditional laboratory diagnostics on a per-test basis, remains a barrier to adoption, particularly in developing countries where agricultural production is most vulnerable to pest and disease losses yet resources for diagnostic technologies are most limited.

Regulatory frameworks for nano-biosensors in agriculture remain underdeveloped in many jurisdictions, creating uncertainty for commercial developers and potential users. Questions regarding the environmental fate and potential toxicity of nanomaterials used in biosensors, particularly if deployed in large numbers throughout agricultural landscapes, require careful assessment. Although many nanomaterials used in biosensors are considered relatively safe, particularly gold nanoparticles and carbon-based nanomaterials, concerns about semiconductor quantum dots containing heavy metals and the potential for nanomaterial accumulation in soils or water bodies necessitate rigorous environmental impact assessments (Holden *et al*., 2014). The lack of standardized protocols for testing nano-biosensor performance under agricultural conditions hampers comparison between different technologies and validation of commercial claims, though efforts are underway through organizations such as ISO and IUPAC to develop such standards.

Current Applications and Case Studies

Despite the challenges outlined above, several nano-enabled biosensors have transitioned from research laboratories to practical agricultural applications, demonstrating the technology's real-world value. In citrus production, where Huanglongbing (citrus greening disease) caused by the bacterium Candidatus Liberibacter asiaticus represents an existential threat to the industry, early detection is critical as infected trees remain asymptomatic for months while serving as inoculum sources (Duan *et al*., 2009). A portable electrochemical biosensor utilizing carbon nanotube-modified electrodes and loop-mediated isothermal amplification (LAMP) for target DNA amplification has been deployed for field testing of citrus trees, providing results in under two hours compared to several days for laboratory PCR testing. The sensor has been adopted by several citrus management programs, enabling more rapid removal of infected trees and slowing disease spread.

In wheat production, nano-biosensors have been applied to detect Fusarium head blight, a fungal disease that not only reduces yield but also contaminates grain with mycotoxins hazardous to human and animal health. An immunochromatographic test strip incorporating gold nanoparticles was developed for detecting deoxynivalenol (DON), the primary mycotoxin produced by Fusarium species, in grain samples (Zhang *et al.*, 2020). The test provides semi-quantitative results visible to the naked eye within 10 minutes, enabling farmers and grain elevator operators to make rapid decisions about grain segregation and use. Thousands of these tests are now performed annually in wheat-growing regions, preventing contaminated grain from entering food supplies. The success of this application demonstrates how nano-biosensor technology, when addressing a clear market need with appropriate performance characteristics and cost structure, can achieve commercial viability.

Greenhouse operations have been early adopters of nano-biosensor technology for continuous environmental monitoring and disease surveillance. A commercial sensor system incorporating multiple nano-biosensors for detecting common greenhouse pathogens including Botrytis cinerea, Pythium species, and various bacterial pathogens has been installed in greenhouse facilities in several countries (Singh *et al.*, 2021). The system provides real-time monitoring of pathogen presence in irrigation water and on crop surfaces, enabling preventive treatments before disease outbreaks occur. The economic benefits of early detection reduced crop losses, decreased fungicide usage, and improved product quality have justified the initial investment in sensor infrastructure for many high-value greenhouse operations. This application exemplifies how nano-biosensor technology is particularly well-suited to controlled environment agriculture where infrastructure can support sophisticated sensor networks and where crop values justify the technology investment.

In developing countries, simple colorimetric nano-biosensors have found applications in smallholder farming contexts where laboratory diagnostics are unavailable. A paper-based test for detecting cassava mosaic virus, incorporating gold nanoparticle-based colorimetric detection, has been distributed to extension agents in several African countries where cassava is a staple crop and virus infection represents a major food security concern (Kumar *et al*., 2020). The test requires no equipment beyond the paper device itself and provides results visible within 20 minutes, enabling farmers to identify infected plants for removal and to source clean planting material. The low cost (under $1 per test) and simplicity of use have enabled widespread adoption, demonstrating that appropriately designed nano-biosensor technology can be accessible even in resource-limited agricultural settings.

CONCLUSION

Nano enabled biosensors represent a transformative technology for detecting and monitoring pests and diseases in agricultural systems, offering unprecedented sensitivity, speed, and accessibility compared to traditional diagnostic methods. The unique properties of nanomaterials high surface areas, distinctive optical and electronic characteristics, and catalytic activities enable biosensor designs that overcome many limitations of conventional diagnostics. Electrochemical nano-biosensors provide sensitive, rapid, and potentially portable detection of bacterial, viral, and fungal pathogens through direct measurement of electrical signals generated by biorecognition events. Optical nano-biosensors, exploiting phenomena such as surface plasmon resonance, quantum confinement, and electromagnetic field enhancement, enable highly sensitive detection with potential for multiplexed analysis and remote sensing capabilities. The extension of nano-biosensor technology to pest detection through monitoring of pheromones, salivary proteins, and stress metabolites expands the scope of these tools in integrated pest management strategies.

FUTURE DIRECTIONS AND EMERGING TECHNOLOGIES

The field of nano-enabled biosensors for agricultural applications continues to evolve rapidly, with several emerging technologies poised to significantly enhance capabilities in coming years. Multiplexed detection platforms capable of simultaneously detecting multiple pathogens or multiple analytes associated with a single pathogen represent a major focus of current research (Dincer *et al.*, 2017). Microfluidic lab-on-chip devices incorporating arrays of nano-biosensors enable comprehensive diagnostic panels from small sample volumes, providing farmers with complete information about the disease or pest pressures their crops face. These devices can integrate sample preparation, amplification, detection, and waste handling in compact formats suitable for field deployment, moving toward the vision of truly portable diagnostic laboratories.

Nanotechnology Applications for Agriculture

Wearable and plant-attached nano-biosensors represent another frontier, enabling continuous monitoring of plant health status at the individual plant level. Researchers have developed nano-biosensors that can be infiltrated into plant leaves or attached to plant surfaces, where they continuously monitor stress hormones, reactive oxygen species, or other biomarkers of pest or disease attack (Lew *et al*., 2020). These sensors communicate wirelessly with external receivers, providing real-time streams of plant health data. While currently at the proof-of-concept stage, such technologies could enable unprecedented precision in agricultural management, with interventions targeted to individual plants rather than entire fields. The integration of these sensors with autonomous agricultural robots for both sensor deployment and responsive treatment application represents a particularly compelling vision for future precision agriculture systems.

Advances in nanomaterial science continue to provide new tools for biosensor development. Two-dimensional materials beyond graphene, including transition metal dichalcogenides such as molybdenum disulfide and tungsten diselenide, offer unique electronic and optical properties potentially advantageous for certain biosensing applications (Choi *et al*., 2017). Plasmonic nanomaterials with increasingly sophisticated architectures including metamaterials with designer optical properties enable new detection modalities and improved sensitivities. DNA nanotechnology provides methods for creating precisely defined nanostructures that can serve as scaffolds for organizing multiple biosensor components with nanometer-scale precision, potentially enabling new multiplexing strategies and enhanced performance through optimized spatial arrangements of recognition elements and transduction components.

The convergence of nano-biosensors with synthetic biology offers intriguing possibilities for creating living biosensors that combine the sensitivity and specificity of biological systems with the signal transduction capabilities of nanomaterials. Engineered microorganisms programmed to produce fluorescent proteins or other detectable signals in response to specific pathogen-associated molecules could be deployed in agricultural environments, with nano-enhanced detection systems monitoring their responses. The integration of CRISPR-based diagnostics with nano-biosensor platforms has already demonstrated remarkable sensitivity for detecting pathogen nucleic acids, and continued development of this approach may provide next-generation diagnostic tools suitable for agricultural applications (Kellner *et al*., 2019). Furthermore, the application of nanomaterials in developing edible sensors that could be applied to fruits and vegetables to monitor ripening, pathogen contamination, or pesticide residues throughout the supply chain represents an emerging application area with significant food safety and quality assurance implications.

Acknowledgement. I extend my heartfelt gratitude and thanks to Principal of Shivaji College, Prof. Virendra Bharadwaj whose constant encouragement and visionary support to work for the academic progress and also his motivation was instrumental to make it success. I sincerely thanks to TIC Ms. Nimita Kant, Department of Zoology, My Colleagues Mr. Bhagat Singh, Dr. Ankita Dua, My Student Abhishek Chaudhary, My Daughter Barsha Priyadarshani and wife Swapna Rani for their unwavering support and constant motivation for progress in academics.

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