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A Paradigm of Internet-of-Nano-Things Inspired Intelligent Plant Pathogen-Diagnostic Biosensors

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Plant pathogens massively affect crop productivity and are one of the significant challenges in attaining sustainable development goals related to agriculture, food production, and addressing hunger issues. Conventional techniques of generic seasonal chemical spraying severely damage the environment and human health. On the contrary, nanomaterials-based biosensors have emerged as economical, efficient, selective, prompt, and precise strategies for plant pathogen and disease diagnosis. The integration of nano-biosensors with artificial intelligence, internet-of-things, cloud computing, drones, and 5G communication has recently raised the paradigm of internet-of-nano-things-inspired intelligent plant-diagnostic biosensors. This prospect highlights these modern-age plant-pathogen biosensors for shaping smart and 5th generation agricultural practices.

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The need to increase agricultural output and ensure the safety of the food supply is rising to meet the demands of a rapidly expanding global population. It is widely accepted that plant diseases contribute to the world's dismal agricultural production. These hostile plant pathogens, including viruses, fungus, and bacteria are seen as disruptive outside influences with the ability to cause a wide range of plant infections and reduce crop yields.¹ The fast spread, growing frequency, and worsening harshness of these threats have caused major worry for the global food supply. It leads to paramount devastation by affecting the crop, which is a staple diet of outsized residents, especially in unwell resourced emerging areas.² Moreover, the Food and Agriculture Organization estimates that plant diseases annually cost the world economy around \$220 billion and offensive insects at least 70 billion. In the U.S. state of Georgia, plant disease and its control loss were estimated in 2019 at around 832 million dollars, total losses due to plant diseases.³

The most conventional used for plant pathogen and resulting symptoms and disease diagnosis is their visual identification necessitating a practiced plant grower or pathologist.^{4,5} However, it is ineffective in detecting pathogens' presence in the early infection phases when plant infections show no outward signs.⁶ There are now three distinct molecular tests that have been used. All of them are based on either proteins or nucleic acids: polymerase chain reaction (PCR), Enzyme-linked immunosorbent assay (ELISA), and other traditional methods like colony counting, fluorescence in situ hybridization (FISH), and flow cytometric detection (F.C.M.) immunology-based approach are used to assess apparatus for pathogen detection.^{7,8} However, these methods are costly, time-consuming, and labor-intensive, hindering the early stage, cost-effective and rapid pathogen diagnosis necessary for diversified crops.

Moreover, significant effort must be made to optimize individual plant monitoring at the site. Immunoassays and DNA-based assays are the two main categories of commercialized methods used to detect and count infections. Several criteria, including the infection's stage and the availability of antibodies and D.N.A. system information (including toxin-producing genes, viral D.N.A., and strain-and species-specific genes), determine whether or not DNA-based tests or immunoassays are used. DNA-based assays are frequently used when conventional antibody tests are inconvenient or inaccurate due to low antibody accessibility, the requirement for susceptible results, or diseases that are unable to produce a considerable degree of

antibody generation in the cell despite the presence of the pathogen. When using a DNA-based test, the pathogen must either be present in the sample or have been there recently. Pathogens may be identified in several ways, including by using antibodies or identifying toxin-generating genes. Therefore, nucleic acids, poisons, cells, oocysts, and viruses are all targets in pathogen detection. Therefore, biorecognition elements such as antibodies, aptamers, and imprinted polymers span a broad spectrum of diversity.^{9,10} There are some drawbacks of immunoassay, which are as follows: assay requires a laborious washing procedure, time-consuming, huge number of Samples, narrow dynamic range, and detection of weak interactions.

Biosensors have emerged as cutting-edge detection techniques used in a wide variety of scientific investigations, from airborne pathogen detection to environmental monitoring to the real-time human blood components detection for the recognition of infections and pesticide in plants and animals. It is a diagnostics device that generates an electrical signal by integrating a biological sensing element and physicochemical transducer when coming into contact with a target analyte or pathogen (Fig. 1). As a result, a transducer takes a biomolecular interaction and turns it into a digital signal. An antibody, D.N.A., enzyme, tissue type, complete cell, etc., may all perform the function of a bioreceptor. The bioreceptor's specialized biochemical interaction gives the biosensor its recognition specificity. A biosensor may be either electrochemical, optical, thermal, or piezoelectric, depending on its transducer. Biosensing approaches for plant pathogen detection have been feasible over the last several decades, with significant diagnostic findings achieved in real-world applications.

The advancements in their sensitivity, detection range, and response time have resulted in the widespread usage of biosensors for agricultural practices in recent years.¹¹ In 2021, their global market valued at \$25.5 (U.S.) billion, and by 2026, that number was projected to rise to over \$36.7 (U.S.) billion, with the medical and health sector being the most prominent segment.³

New-generation Biosensors

Recently, the nanomaterial's contribution to enabling sensing of pathogens in plants and crops has covered various transduction mechanisms. Due to their unique properties, such as a high surface-to-volume ratio, the ability to tailor their shape, size, arrangement, and composition, and the flexibility to modify their surfaces with a wide variety of molecular ligands, nanomaterials have garnered a lot of attention for use in biosensing applications. Since the detection of plant pathogens often involves quantifying target analytes at low

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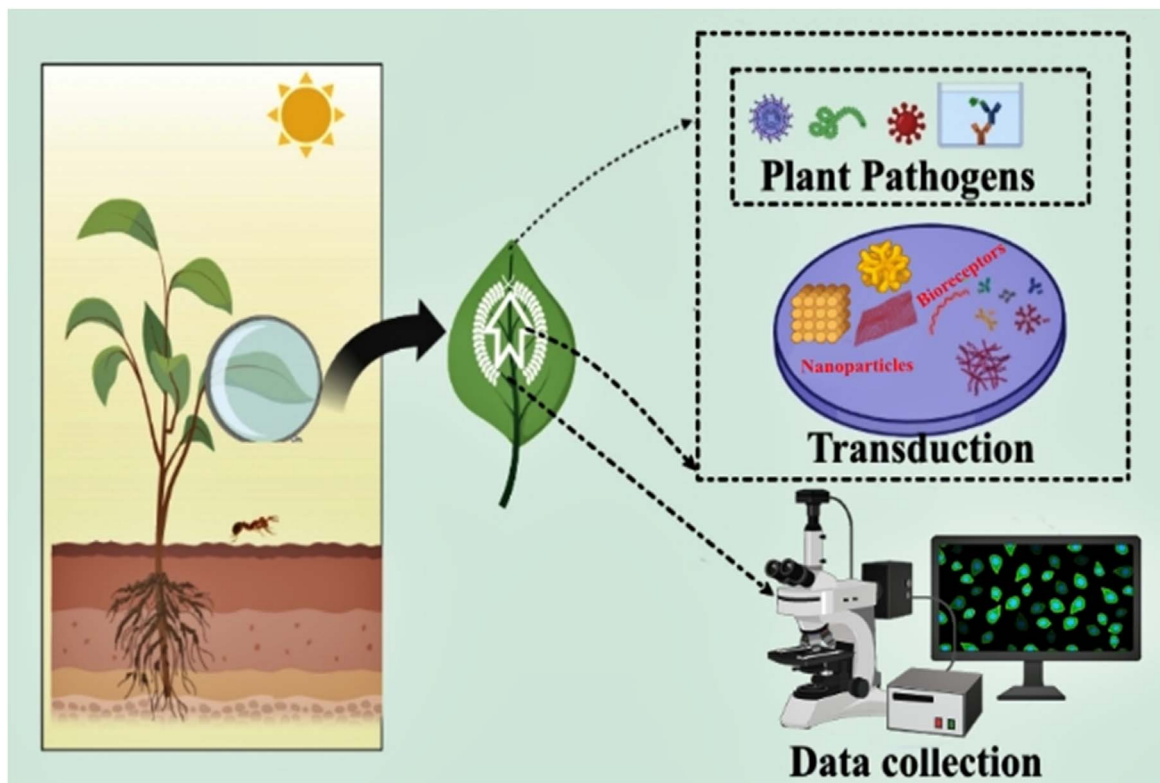


Figure 1. Plant pathogen detection using biosensors and data collection with the latest technology.

concentrations and in complicated matrices, these characteristics are crucial in designing high-performance biosensors. Due to the nanomaterial's quick reaction times and developments in the design of tiny and portable sensors, remote and on-site plant health monitoring is much improved, allowing for the estimate or early detection of plant illnesses. These characteristics, combined with a wide range of optical and electrical properties, provide promising optical, electrochemical, and electronic sensing systems. Integrating nanotechnological tools has revolutionized plant biosensing strategies to detect and monitor a diversified range of plant pathogens.^{12–14} For instance, various nanostructures can be used to create biosensors with enhanced performance in terms of sensitivity, selectivity, and limit of detection, opening the door to manufacturing devices for on-site detection.¹⁵ Binding sites for bio-specific immobilization can be achieved using electrospun nanofibers or metallic nanoparticles, which can alter their surface functionality. Molecularly imprinted polymer (M.I.P.) allows the sensor's selectivity to be fine-tuned by interacting only with the target analyte and blocking out noise. These cases show the potential of nanotechnology's flexibility, which may be used to create biosensors with superior performance.^{16,17,18} It is becoming clear that devices based on nanomaterials-based biosensors provide a good replacement for the conventional, time-consuming techniques of identifying infections (Table I).

Depending upon the detection signal, the biosensors can be classified as electrochemical, optical, acoustic, calorimetric, mass sensitive, and electrical biosensors. An electrochemical biosensor consists of a working electrode, the primary component of most electrochemical cells, and serves as the electrochemical biosensors' transduction element. Potentiostatic systems typically need a set of three electrodes (working, auxiliary, and reference), whereas conductometry and electrochemical impedance spectroscopy often only require two electrodes (working and auxiliary) (E.I.S.). A charge is transferred from one electrode to another by the flow of electrons and holes.³⁹ For this reason, electrodes are made from metals like gold (Au) and nonmetals like carbon, which are both conductive and

semiconducting in electrical nature. Therefore, electrodes may be categorized based on the materials used, fabrication technique, and their architect. Planar, wire, nanostructured, and array-based electrode designs are all possible.⁴⁰ The performance of a biosensor, including its selectivity, sensitivity, dynamic range, and the limit of detection (L.O.D.), is determined by the structure and characteristics of the electrode, which in turn are affected by the material manufacturing process, and design.⁴¹ Metals are often deposited on insulating surfaces using conventional microfabrication techniques like physical vapor deposition and screen printing to create thin-film metal electrodes. The final transducer element is usually completed by embedding the conducting components in an insulating polymer or ceramic substrates such as polyether ketone (P.E.K.), Teflon, or glass. Pathogen detection using conducting and semiconducting ceramics has also been investigated. These materials include polysilicon, indium tin oxide (I.T.O.), and titanium dioxide (TiO₂). Pathogen-detecting electrodes made of polymers have also been studied.⁴² Bio-recognition element immobilization methods that work with polymer electrodes are also available. Implantable and wearable biosensors benefit significantly from having mechanical qualities that allow for electrode-tissue mechanical matching, and polymers provide this.⁴³

For electrochemical biosensors, biorecognition components may be classified as either (1) biocatalytic or (2) biocomplexing.⁴⁴ Enzymes serve as biorecognition components in a wide range of biochemical sensing applications; however, they are usually introduced as labels in the recognition of pathogen settings, typically via secondary binding stages.^{45,46} When analytes interact with macromolecules or ordered molecular assemblies, biocomplexity biorecognition components trigger a response in a biosensor.⁴⁴ The assay design, biosensor performance (e.g., sensitivity and L.O.D.), volume, material qualities, the composition of the pathogen-containing sample, and the application all play a role in determining the optimal measurement format. Functionalized electrodes may be used in various electrochemical procedures, allowing for the detection of pathogens. The measured signal is often used to classify the

Table I. Nanomaterial-based biosensors for plant pathogen detection.

Material	Pathogen	Biorecognition element	Method	Detection limit	References
ITO electrode	<i>E. coli</i>	Monoclonal Anti-E. coli	EIS, CV, Fe(CN) ₆ ^{3±}	4 × 10 ³ CFU ml ⁻¹	19
Au electrode	<i>E. coli</i>	Polyclonal Anti-E. coli	EIS, CV, Fe(CN) ₆ ^{3±}	50 CFU ml ⁻¹	20
Au electrode	<i>S. typhimurium</i>	Polyclonal anti- <i>S. typhimurium</i>	Fe(CN) ₆ ^{3±} , EIS	10 CFU ml ⁻¹	21
Au microelectrode	<i>S. typhimurium</i>	Anti- <i>S. typhimurium</i>	Fe(CN) ₆ ^{3±} , EIS	500 CFU ml ⁻¹	22
graphite interdigitated microelec- trode array	<i>E. coli</i>	<i>E. coli</i> -specific bacteriophages	EIS	10 ⁴ CFU ml ⁻¹	23
nanostructured alumina on Pt wire electrode	West Nile virus	monoclonal anti-WNV	AC voltammetry	0.02 viruses ml ⁻¹	24
CNTs on carbon rod electrode	<i>S. typhimurium</i>	Anti- <i>S. typhimurium</i> aptamer	Potentiometry	0.2 CFU ml ⁻¹	25
CNTs on carbon rod electrode	<i>E. coli</i>	Anti- <i>E. coli</i> aptamer	Potentiometry	6 CFU ml ⁻¹	26
Ag electrode	<i>B. anthracis</i>	monoclonal and polyclonal anti- <i>B. anthracis</i>	conductometry	420 spores ml ⁻¹	27
AuNPs on nickel foam electrode	marine pathogenic sulphate-reducing bacteria (SRB)	anti-SRB	EIS	21 CFU ml ⁻¹	28
Ag nanofiber array electrode	bovine viral diarrhea virus	monoclonal and polyclonal anti- BVDV	Conductometry	103 CCID ml ⁻¹	29
nanostructured alumina on Pt wire electrode	dengue type 2 virus	monoclonal anti-DENV-2	DPV; Ferrocene methanol	1 PFU ml ⁻¹	30
CNT/polyallylamine composite on graphite electrode	Campylobacter	monoclonal anti- <i>Campylobacter</i>	ASV	400 cells ml ⁻¹	31
polypyrrole nanoribbons on Au mi- croelectrode array	cucumber mosaic virus	polyclonal anti-CMV	Amperometry	10 ng ml ⁻¹	32
reduced graphene oxide microelec- trode	rotavirus	anti-rotavirus	Amperometry	100 PFU	33
AuNPs microelectrode	avian influenza virus (AIV) H5N1	anti-AIVH5N1, concanavalin A lectin	CV	0.367 HAU ml ⁻¹	34
carbon electrode	human influenza A virus H9N2	polyclonal anti-influenza A virus M2 protein, fetuin A melittin peptide	chrono-ampero- metry	16 HAU	35
Ag interdigitated microelectrode array	<i>E. coli</i>		EIS	1 CFU ml ⁻¹	36
Gold nanoparticle based	Banana bunchy top virus	DNA	lateral flow assays	3.2 × 10 ⁴ pg ml ⁻¹	37
Gold nanoparticle based	Acidovorax avenae subsp	DNA	lateral flow assays	7.25 × 10 ⁴ pg ml ⁻¹	38

electrochemical techniques used for pathogen detection. These techniques may be categorized as amperometric, conductimetric, potentiometric, ion-charge/field-effect, or impedimetric. Signals may be applied either continuously or intermittently.⁴⁷ Steady and transient reaction analysis or a mix of the two may be necessary to understand the electrochemical method's output signal fully. Nguyen et al. demonstrated that Using antiWNV-DIII immunoglobulin M (IgM) as the biorecognition probe, we show that our nanobiosensor can detect both the native and inactivated forms of the West Nile virus protein domain III.²⁴ The recognition of the virus-related element and protein are logarithmically linear up to 50 viral elements per 100 ml⁻¹ ($R^2 = 0.93$) and 53 pg ml⁻¹ ($R^2 = 0.99$) in pH7, with tremendously low revealing limits of ca. 2 viral particles per 100 ml and 4 pg ml⁻¹, comparable to sensitivities of polymerase chain reaction techniques. An RSD of 6.9% is found while testing for viral RNA during the whole serum sample. Wan et al. fabricated reduced graphene sheets with doping of impedimetric immunosensor for bacteria detection.²⁸

An optical biosensor includes a light source, a medium for transmitting light, a biorecognition element that has been immobilized, and a signal detection mechanism. Together, they help determine the strength of a given analyte's binding to a certain ligand. The biorecognition process generates a physicochemical conversion (change), which is quantified by measuring the resulting light's amplitude, phase, and frequency.⁴⁸ Colorimetric biosensors, biosensors based on the surface plasmon resonance, and fluorescence-based assays are the most prevalent types of optical biosensors designed for plant pathogen detection.⁴⁹ Colorimetric biosensors often enable the user to identify harmful bacteria in a few samples in as little as 10–15 min by observing a color change in the samples. This assay may be performed in either a flat-based or solution-based format.⁵⁰ All these modules of biosensors based on nanomaterials have been utilized to detect various plant pathogens and diseases as summarized in Table I. Wahabzada and coworkers developed a machine learning approach to characterize hyperspectral reflectance signals across net blotch, powdery mildew, and brown rust pathogenesis in barley.⁵¹ This has the potential to pave the way for a new genre of spectral libraries that detail the illnesses and health of plants via their spectral properties. Accumulating hyperspectral signatures of plants would have far-reaching benefits and provide opportunities for others who are not trained in the field. Investigation by Pinto and coworkers used hyperspectral imaging to examine the solar chlorophyll fluorescence and develop a technique to measure the Spatio-temporal dynamics of the illumination of plant canopy and photosynthesis activities.⁵² By doing so, we may evaluate how well photosynthesis is occurring in plant canopies. However, spectral pictures also need to be related to biological processes, notably during plant-pathogen interactions.

When compared to recognition elements based on antibodies for bacterial detection, aptamer (single-stranded nucleic acids) have many advantages, including being chemically stable and cost-effective.⁵³ The “chemical nose” method has just lately become standard detection gear for infectious diseases.⁵⁴ It assigns several selective receptors, each of which produces a distinct response configuration, allowing the objectives to be ranked. Like how our brains process odors, it works in a manner similar to how it is perceived.⁵⁵ Bacterial pathogens are identified by cross-referencing them against a standard reference collection. Nanoparticle-based “chemical nose” biosensors often call for the modification of the nanoparticle's surface with many ligands, where each ligand is responsible for a unique communication with the goal.⁵⁶ Because each pair of particles may respond to various kinds of bacteria uniquely, this variation in size and exterior make-up is purposefully chosen to add complementary aspects to the absorption spectrum.⁵⁴ Electrostatic interactions between the anionic parts of the bacterial cell walls and the cationic CTBr (cetyltrimethylammonium bromide) are responsible for the formation of aggregates around the bacteria after their addition to nanoparticles. As the particles get more concentrated, a shift in localized surface plasmon resonance occurs,

causing a corresponding color shift. In the presence of many bacteria, obtaining an absorption spectrum also serves to signify color variation.^{57,58} The presence of extracellular polymeric molecules on the bacterial surface motivates these distinctive aggregation patterns. Distinct colorimetric responses may be attributed to these varied aggregation patterns.⁵⁹ In this way, the “chemical nose” developed on nanoparticles might be used to detect mixtures of different bacterial species. The “chemical nose” is sensitive enough to distinguish between monomicrobial and polymicrobial infections, allowing for more rapid antibiotic treatment and better outcomes without requiring lengthy sample testing. The very sensitive multi-channel nanosensors can detect different types of bacteria, including those living in biofilms, in a matter of minutes.

Integration of Modern-age Technologies

A newly developed service, such as Cloud-IoT, enables a no-cost web service that facilitates the plug-and-play installation of remotely located sensors. Since farmers have started using new techniques that have enhanced crop yields, farming has become more accessible thanks to technological advances. The IoTs contains sensors, robots, mobiles and drones linked over the internet to work automatically and semiautomatically to execute many functions and gather data to enhance productivity and accuracy. Artificial intelligence (A.I.) tools are used in “smart farming” to monitor and analyze a farm's environment to make informed planting and crop selection decisions based on factors such as soil type, weather forecasts, water availability, and more.⁶⁰ To back up the 5G communication network, a simple channel is provided to link the gadgets for data transactions without traffic, loss, or delay; this drastically cuts down on time, effort, and money. As for download and upload speeds, 5G will be up to one hundred times faster than 4G and 4G LTE. Uncrewed aerial vehicles, predictive maintenance and virtual consulting, real-time monitoring, AI-powered robotics, augmented and virtual reality (AR/VR), cloud repositories, data analytics, and more are all described as potential uses of 5G in plant pathogen detection. With 5G technology, it's much simpler to set up, track, and administer a vast network of IoTs devices and plant pathogens.

In agriculture, 5G will be beneficial for integrating vast volumes of data from several sources. The temperature and feeding equipment readings on a large-scale chicken farm are sent to a single hub. The data generated by these thousands of sensors is too little to justify the expense and complexity of a 5G broadband connection. Bandwidth may be in line with 5G mobile broadband bandwidth if nodes are aggregated in clusters of the right size.⁶¹ Drones equipped with sensors and multispectral cameras collect data as they fly over farms, which is then used by computers running deep learning algorithms to assess and diagnose plant problems. They will have the ability to record reflected light from certain sections of the electromagnetic spectrum using specialized filters, as shown in Fig. 2.

Many farmers are relying on drones for crop monitoring. Cost-effectively assessing crop damage and other factors is possible using drones rather than sending tractors over fields. Drones can capture and transmit video footage at a better quality and at a quicker rate because of the 5G capacity to handle large amounts of data.⁶² Plants that are under stress generally have a different “classification” than plants that are not under stress. This can be easily taken by using IoNT-integrated biosensors with the assistance of AI, drones, 5G communication, and machine algorithms. These concerns, if given strategic consideration, will aid in the development of superior nanosensor products and their eventual incorporation into the agricultural ecosystem. Razzberry, a company based in the United States, created handheld chemical nanosensors to supervise chemical shifts in water and soil in real-time. Nasys, an Italian startup, also developed a nanosensor based on metal oxides to measure air pollution. Startups like Tracense and nGageIT are developing nanosensor innovations to trace harmful biological and chemical contaminants in food production.⁶³

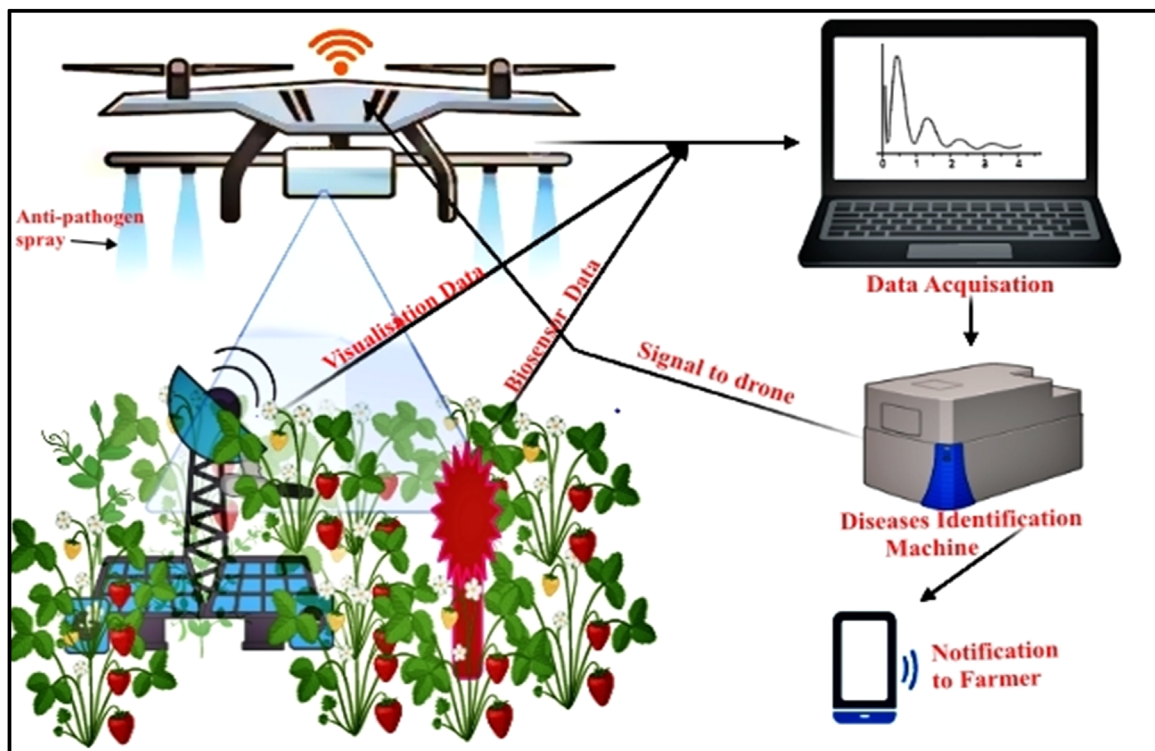


Figure 2. Plant pathogen detection using drones powered by 5G network.

Future Needs and Prospects

Many other factors within each farming system must be considered in addition to pathogen detection and quantification data. Developing reliable, cost-effective biosensors for detecting pathogens is a significant issue. Recent advancements in nano- and micro technologies have allowed the creation of plant-based biosensors that are extremely sensitive, fast, and selective. Recent interest has been in exploring carbon-based electrodes (e.g., graphite, graphene, C.N.T.s) as prospective substitutes for the comparatively more costly metallic or ceramic electrodes. It's worth noting that many of these carbon-based materials have benefits in terms of nanostructuring since they have nanoscale structures. There are some challenges that we have to overcome nanomaterials may be toxic, genetically modified due to a combination of plants, and generation of e-waste due to the use of IoT, and slow detection time. Alternative for these challenges is: toxicity can be reduced by functionalizing nanomaterials with biocompatible materials like P.E.G., green synthesis can be used in the fabrication of nanomaterials, use of the A.I. technique to know the interaction between nanomaterials and plant to observe genetic modification, and use of biocompatible materials to form sensors repurpose, reuse, degradable or recyclable material. The sustainable development goals that can be achieved through IoT nanomaterial-based biosensors are smart farming, reduction of human resources, preservation of crops, and food chain. Manufacturing cost reductions for biosensors have been studied alongside attempts to minimize the per-device material cost. It is significant to note that the performance of a particular electrochemical biosensor may be significantly affected by the size of the pathogen, depending on the specific electrochemical technique used. But there are many other factors to think about potential challenges to implementing them widely in plant pathogen detection and monitoring. Although biosensors are expected to become a standard component of integrated disease management (I.D.M.) programs, they must be thoroughly validated for use within specific plant diseases. Using them to make disease management decisions requires reliable background information on plant phenology, pathogen biology, and disease epidemiology. Pathogens, for

instance, may vary in size by more than three orders of magnitude. In recent years, multiplexed recognition of pathogens has evolved as a method for instantaneously recognizing phenotypes and numerous pathogenic risks. While there are several methods for accomplishing multiplexing, one of the most common ones includes employing numerous transducers with unique biorecognition features. Biosensor-based procedures for control and monitoring applications face significant challenges due to the inability to renew biosensors. Biosensing technologies for recognizing pathogens and remote sensing technologies for disease control and plant monitoring would considerably benefit from investigating wireless transduction and monitoring technologies. To create injectable and linked biosensors for the detection of pathogens, particularly those utilized for diagnostic purposes, wireless biosensing platforms are essential.

Conclusions

In order to learn precision agriculture, it is necessary to keep a close eye on a variety of environmental factors and then take the appropriate course of action. Internet-of-nano-things-inspired biosensors with the assistance of drones, AI, and cloud computing are rapidly transforming the traditional methods of plant-disease diagnosis. By properly identifying the kind and location of hiccups, this monitoring even helps define the development of agricultural plants. In the end, it makes use of smart sensors to provide precise data that grants heightened production by assisting farmers in making sensitive recovery decisions. Smart nanosensors, in particular, have begun to prove themselves to be indispensable instruments for supporting agricultural sustainability because of their high sensitivity and their judicious use. Researchers have shown that biosensors and nanosensors may increase crop yields. These real-time sensors are able to detect changes in soil temperature, soil health, soil moisture content, and the soil's microbiological state and nutritional status. Pesticide residues, heavy metals, plant diseases, fertilizer, toxin quantification, and monitoring have all been detected by these sensors. The data gathered by these nanosensors may be used to forecast and prevent crop failures in agroecosystems. In addition, biosensors based on nanotechnology are helping bring the

notion of sustainable agriculture to life. It has been noted that advancements in sensitivity and specificity of nanosensors and/or biosensors are necessary for their projection as plant diagnostic instruments. Multiplexed screening that can identify a large variety of plant-based bioproducts quickly, reliably, and affordably is also necessary. Technology mobilization will also benefit from the development of multi-entity detectable, broad-spectrum nanosensors. Some have speculated that in the not-too-distant future, we may be able to create super “new nanomaterials” that will vastly increase the performance of biosensors.

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