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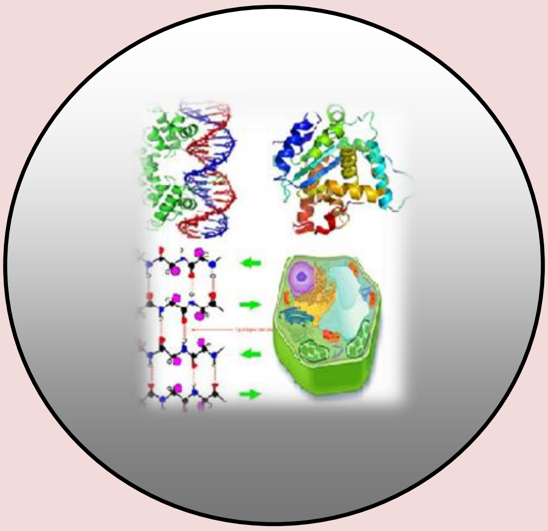

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Applications of Nanotechnology in Diagnosis of Plant Diseases

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ABSTRACT
Plant diseases caused by pathogens like bacteria, viruses and fungi adversely affect crop productivity. Correct diagnosis of phytopathogens is very helpful in management of plant diseases. Therefore cost-effective and highly sensitive methods for early detection of plant diseases and pathogens are needed for minimizing damage caused by various plant diseases in crops and to ensure sustainable agricultural productivity. Advance and efficient crop protection methods to stop crop damage can increase agricultural productivity and thus improve the global food security scenario. Traditional diagnostic techniques are time consuming, lack high sensitivity and they are not useful for adaptation to high throughput technologies. Therefore development of cost-effective, accurate and rapid methods for detection of phytopathogens is need of the present time. Nanotechnology is a recent interdisciplinary technology combining different branches like science, technology, medicine and agriculture. Nanotechnology can be used for developing advance diagnostic methods capable of rapid and early detection of pathogens with ultra sensitivity and specificity and can be potentially used for large-scale analysis. In current review various applications of nanotechnology for developing rapid, cost-effective and highly precise techniques for detection of plant pathogens are discussed.
Key Words: Detection, Food Security, Nanosensors, Nanotechnology and Plant Pathogens.

INTRODUCTION
Agriculture has played a significant role in development of many nations. Agriculture is at centre stage of national economy particularly in developing countries and it has been a source of income for a sizeable portion of population in these countries (Brock et al., 2011). But in recent times productivity of agricultural crops has been shown to be reduced. The main reasons stated for this are reduction of agricultural land, climate change and crop damage caused by pests and pathogen infections, which have globally affected food production in a negative way and resulted in enhancement in food prices due to gap between their demand and supply and subsequently malnutrition (Ingram, 2011; Savary et al., 2012). This global food scenario of disparity between demand and supply can be further worsened by increase in global population in coming times (Naderi and Shahraki, 2013). Post-cultivation crop damage caused due to aftereffects of diseases and post-harvest malpractices are also responsible for crop losses globally.

Efficient and rapid disease diagnosis methods in crops are need of recent times to decrease crop loss due to pathogen infection, which can improve global food scenario by increasing agricultural productivity. Diagnosis of crop diseases at an appropriate time at which disease can be controlled and managed effectively is very important. Late application of pesticides and other disease control agrochemicals and strategies can not efficiently control the disease, once it has progressed to an advanced stage and it also adds towards the cost of disease control and management and crop loss (Sharon et al., 2010).

Conventional methods for diagnosis and identification of plant pathogens are time taking and not very sensitive towards the pathogens and biochemicals produced by them. Therefore, extremely sensitive, rapid detection diagnostic tools at molecular and biochemical level are required in recent times (Misra et al., 2013). Many traditional molecular diagnostic methods such as polymerase chain reaction (PCR) and biochemical methods are frequently used in laboratories for disease diagnosis. These methods, although sensitive and specific, but they are not fit for farmer's field application especially in developing countries because they require high technical expertise and they are not cost-effective **(**Khiyami et al., 2014).

Nanotechnology is a comparatively recent scientific development which is exerting huge influence in many aspects of our lives. The concept of Nanotechnology was put forward by Richard Feynman, Nobel Prize recipient in Physics in 1965 (Feynman, 1960; Hulla et al., 2015). The word "Nanotechnology" was brought into public domain by Norio Taniguchi (1974). Nanotechnology has been developed by combining knowledge from areas as diverse as physics, chemistry, biology, material science and engineering (Roco, 2007). The term "Nanotechnology" has been developed from the prefix of Greek word "nano" which corresponds to 10^{-9}. According to one definition of nanotechnology given by National Science Foundation (NSF, USA) and National Nanotechnology Initiative (NNI); "Nanotechnology is the understanding and regulation of matter at measurements between approximately 1 to 100 nanometers (nm) where unique phenomena involved novel applications" (Misra et al., 2013). Many excellent properties of nanoparticles like nanometer range dimensions (1-100nm), higher surface area, enhanced surface reactivity and catalytic activity (Ghormade et al., 2011) make them ideal material for many novel applications. Nano-phytopathology is a newly emerged field which employs nanotechnological principals for detecting, identifying and monitoring plant diseases and phytopathogens swiftly at an early stage, so that massive crop and economic losses can be stopped (Khiyami et al., 2014). In this review, the concepts and recent nanotechnological applications in development of diagnostic methods for plant pathogen and disease detection are discussed.

Nanotechnology for detecting phytopathogens

Need for diagnosis methods that are rapid, specific with light, portable instruments and simple operation and capable of early on-site diagnosis, motivated scientists to consider nanotechnology for developing diagnostic tools and techniques for detection of various phytopathogens. Amongst these nanotechnology based diagnostic techniques like nanosensers, quantum dots, nanobarcodes, nanobased kits and other portable diagnostic instruments have the potential for early, cost-effective detection and regulation of various plant diseases **(**Khiyami et al., 2014).

Nanosensors in diagnosis of phytopathogens

Nanosensors work on chemical or mechanical interactions which may be employed to detect the prescence of chemicals and nanoentities, or measuring physical aspects such as temperature, at the nanolevel (https://www.nature.com/subjects/nanosensors). In naosensors, the mode of detection of contaminants can be through sensing based on electrical, chemical, electrochemical, optical, magnetic or vibrational signals. Nanosensors being miniature in size are portible, they are sensitive, precise, have capability of real time monitoring and they can sense a wide variety of biological and chemical infectious agents and in this aspect show superiority to other type of sensors. Nanosensors have applications in areas as diverse as environment, agriculture and medicine (Fang and Ramasamy, 2015). Nano sensors in conjuction with a GPS (Global positioning system) and The Nano-Sensor for Controlled Environmental Agriculture (CEA) can monitor plants especially crops in real-time which will be very helpful in detection of plant diseases and phytopathogens at the earliest and thus will be very much useful in disease control and management and preventing further crop losses (Misra et al., 2013; Agrawal and Rathore, 2014).

Nanobiosensors

A biosensor is a measurable sensing instrument containing bio-recognization elements as probes and it is constructed for estimation of an analyte by employing the biological reactions and then converting these into an interpretable form using a transducer and electromechanical interpretation. The main parts of a biosensor are, bioreceptor, transducer and the detector. The biological entities (analytes) assessed by biosensors usually are biochemicals like antibodies, proteins, enzymes; micro-organisms such as plant pathogens themselves, tissues etc. (Malik et al., 2013). The mode of operation of a biosensor involves the response of biorecognization element to the analyte and then transducer changes the resultant biological interactions into assessable signals, which may be analyzed by various methods like electrochemical, optical, mechanical, calorimetrically or electronically, and then correlated with the target concentration (Schubert et al., 1991; Wilson, 2005). The nanobiosensor, a recent technology is made up employing principles of biology and nanotechnology (Yang et al., 2008). Nanobiosensors because of addition of biological sensing probes are more specific as compared to nanosensors without biological elements. Nanobiosensors are sensitive and may be employed for rapid and early detection of various plant diseases (Small et al., 2001).

Nanomaterials employed in biosensors

The preference of nanomaterials for bio-sensor manufacturing may be due to the suitability of nanomaterials as an immobilization platform for putting biological sensing probes, enhanced surface area and surface activity and enhanced electronic conductivity that contribute towards increasing the detection limit of biosensors. The nanomaterials and nanostructures employed for biosensor assembly include metal and metallic oxide nanoparticles, carbon nanomaterials like carbon nanotubes and graphene, silicates and polymeric nanomaterials, quantum dots and nanofibres (Fang and Ramasamy, 2015). Carbon nanotubes employed in nanosensors, help in capturing individual proteins and small molecules which are measured by suitable methods (Sharon et al., 2010). Besides these, there are reports of other nanomaterials employed in biosensor construction for ultrasensitive detection (Kuila et al. 2011; Pérez-López and Merkoçi, 2011; Shiddiky and Torriero, 2011).

Categories of biosensors

Various versions of biosensors in use recently are affinity sensors, metabolism sensors and catalytic sensors. Amongst them, affinity biosensors are used frequently (Schubert et al., 1991; Wilson, 2005). In affinity biosensors, interaction between the target analyte molecule and bio-recognition probe is the basic sensing method (Sadanandom and Napier, 2010). The bio-recognization elements employed in manufacturing affinity biosensors are antibody (Yao et al., 2009; Perdikaris et al., 2011), DNA (deoxyribonucleic acid), (López et al., 2009; Kumar et al., 2015), enzymes (Thomas et al.,1999; Ronkainen et al., 2010) and bacteriophages (Schofield et al., 2013; Frampton et al., 2014).

Gold nanoparticles in development of biosensors

Recently DNA-or protein functionalized gold nanoparticles have been used as probes for detection of analytes (Fan et al., 2003; Cao et al., 2011). Gold nanoparticles have the property of quenching the fluorescence of light harvester polymers and therefore they have potential to be employed in construction of optical technology based sensors for detection purposes (Fan et al., 2003). Gold nanoparticles have been employed in the detection of the phytoplasma linked with flavescence doree (FD) of grapevine (Firrao et al., 2005). Sensing of methyl salicylate, a major phyto-volatile organic compound (VOC) released by plants in response to phytopathogen infections was done with probes involving gold nanoparticles (Umasankar and Ramasamy, 2013).

Semiconductive metallic oxide nanoparticles were employed for VOC detection because of their comparative cost-effectiveness and ease of use. Different types of metallic oxide nanoparticles likeTin dioxide (SnO_2) and Titanium dioxide (TiO_2) have been employed for VOC detection in infected strawberry (Fang et al., 2014). Copper oxide (CuO) nanoparticles were also used for detecting the *Aspergillus niger* fungi (Etefagh et al., 2013). Besides detection of VOC released by plants during pathogen infection, nanoparticles can also be employed for detection of chemical compounds released by the phytopathogen itself (Boonham et al., 2008; Yao et al., 2009).

Quantum dots in disease detection

Quantum dots (QDs) are fluorescent nanoparticles of semiconductors that fluoresce because of stimulation by a light source (Arya et al., 2005). QDs were employed as a material for manufacturing of biosensors for disease detection (Frasco and Chaniotakis, 2009). Role of QDs in biological detection was first reported in 1998 (Chan and Nie, 1998). Some QDs were created by mixing suitable fungal species with inorganic compounds and incubating them at suitable temperatures. Examples are cadmium-selenium (CdSe) QDs created by incubating the fungus *Fusarium oxysporum* with combination of cadmium chloride ($CdCl_2$) and selenium tetrachloride ($SeCl_4$), (Kumar et al., 2007) and cadmium-tellurium (CdTe) QDs by mixing the *F. oxysporum* with combination of $CdCl_2$ and tellurium dichloride ($TeCl_2$), (Jain, 2003). QDs have been employed for disease diagnosis with a mechanism called fluorescence resonance energy transfer (FRET) (Algar and Krull, 2008), which is energy interchange between two optically-sensitive compounds. Sensors involving QD-FRET technology were employed for detection of witches' broom disease of lime (WBDL) resulted by the infection of phytopathogen *Candidatus Phytoplasma aurantifolia* (*Ca. P. aurantifolia*) and also for detection of vectors of some phypathogens (Safarpour et al., 2012). Advantages of QDs are their optical sensitivity, photo stability, they are user friendly and their capability of early detection of diseases (Sharon et al., 2010).

PCR instruments suitable for on-site disease diagnosis

Different portable PCR systems have been developed by different companies which may be suitable for field detection and idenfication of various phytopathogens. Examples of portable PCR systems are Palm PCR of Ahram Biosystems Company (Korea) and Twista, a portable PCR system **(**Khiyami et al., 2014).

Loop-mediated isothermal amplification (LAMP-PCR)

Loop-mediated isothermal amplification (LAMP) of DNA, (LAMP-PCR of DNA) is a comparatively low cost rapid, diagnosis method for analyzing genomic DNA at field level, which can amplify the analyte sequence specifically (Notomi et al., 2000). This technique employs six primers having eight binding sites hybridizing to various areas of an analyte gene, and a thermophilic DNA polymerase of *Geobacillus stearothermophilus* for amplification of DNA of *Fusarium graminearum* (Niessen and Vogel, 2010; Abd-Elsalam et al., 2011). LAMP-PCR was observed to having potential of monitoring inoculum concentrations in the air and prediction of pathogens in the field before visible symptoms appear. LAMP-PCR products can be seen directly with naked eye or through ultraviolet trans-illumination **(**Khiyami et al., 2014). LAMP technique may be combined with reverse transcription technique to allow identification of ribonucleic acid (RNA). LAMP-PCR technique diagnosed pathogenic fungi *Phytophthora sojae* causing Soybean *Phytophthora* root rot (Dai et al., 2012), toxigenic fungi *Aspergillus flavus*, *A. parasiticus* (Luo et al., 2012) and *Fusarium* (Niessen and Vogel, 2010; Abd-Elsalam et al., 2011; Denschlag et al., 2012).

Nanopore genome sequencing technique: MinION

Nanopore sequencing technology is being considered by many companies as next generation sequencing technology (Niedringhaus et al., 2011; Ozsolak, 2012). Nanopore sequencing technology gives good amount of sequencing data but requires low quantity of target nucleic acid (Rai and Ingle, 2012). A nanopore is a very small nano-scale hole. An ionic current is discharged to nanopores. Changes in current are analyzed and measured (though sensor chips) as single biomolecule pass through the nanopore (Clarke et al., 2009). Companies like International Business Machines (IBM) and Roche together have worked on developing a new sequencing technology by combining genetics with semiconductor technology. This technology was called as 'DNA transistor' which could potentially generate large nucleotide sequences rapidly (Zhang et al., 2011). Another sequencing technology company, Oxford Nanopore Technologies Limited is an organization in United Kingdom which is developing and marketing nanopore sequencing products, used for obtaining rapid sequencing data of individual molecules. Through Oxford Nanopore Technologies, a genomic map in very short time at low cost can be produced. MinION, the pioneer commercial sequencer employing nanopore method, was conceptualized by Oxford Nanopore Technologies. MinION is the portable, real time monitoring next generation, cost-effective, rapid genome sequencing technology for RNA and DNA sequencing.

It can sequence 10 kb of a single sense and anti-sense DNA strand. MinION device can be plugged into a Laptop or personal computer (PC) for real time analysis. MinION may be useful for targeted sequencing and epigenetics. Nanopore sequencing technology in conjunction with latest diagnostic instruments can analyze and generate very large genome data in a short time (Lu et al., 2016).

DNA bio-barcode test, nanodetection kit and nano-imaging

The bio-barcoded DNA (b-DNA) technology is a highly specific assay for swift detection of biomolecules like proteins (Goluch et al., 2006) and nucleic acids (Nam et al., 2004). It employs oligonucleotide-modified magnetic gold nanoparticles (AuMNPs). In nanodetection kit, all components of a disease diagnosis system are packed in a portable structure like a briefcase or parcel-like structure (Goluch et al., 2006). The main advantage of the nanodaignostic kit is its portability, on-site use at field, cost-effectiveness, rapid detection and ease of use. Farmers may themselves use the kit after some training. For example, 4-mycosensor is an antibody-based assay in a portable device for the detection of multiple mycotoxins simultaneously in corn, wheat, oat and barley samples (Lattanzio et al., 2012). In nano-imaging technology, diagnosis of different plant pathogens is done through ultrasensitive imaging of diseased phyto-tissues which is helpful in specific and early analysis of plant diseases before visible disease symptoms appear on infected plants and it could save significant crop losses **(**Rosen et al., 2011). The mechanism of infection of bacterium responsible for Pierce's disease inside grapevine tissue was studied using nanoimaging methods which can lead towards development of methods for control and prevention of disease (Meng et al, 2005).

CONCLUSIONS

People can benefit from many uses of nanotechnology in areas as diverse as environment, medicine and agriculture. Nanotechnology can contribute at large-scale for development of equipments for rapid, early detection of phytopathogens at field level. There are many techniques for plant disease detection including conventional methods and traditional polymerase chain reaction based diagnostic methods, but these methods have limitations mainly because they are not suitable for cost-effective, early, on-site diagnosis of phytopathogens. Different nanotechnology based methods like nanosensors, quantum dots, portable PCR and sequencing systems, biobarcoded DNA and mobile nanodiagnostic kits have been useful for quick, precise, early and field level detection of plant diseases and thus preventing crop damage through prevention and control of diseases and subsequently preventing economical losses to farmers. Different types of nanomaterials are used for nnaosensor construction which includes metal based nanoparticles, carbon nanomaterials like carbon nanotubes as well as polymeric nanomaterials. The nanobiosensors are developed by inclusion of bio-recognization elements like antibody, DNA, enzymes, bacteriophages in nanosensors. These biorecognization elements provide more sensitivity and specificity to nanobiosensors, although each of these elements have benefits and limitations. Naobiosensors due to their increased specificity could diagnose phytopathogens at an early stage before visible disease symptoms appear and thus they can help in prevention of crop losses and improve food safety scenario in agriculture. Gold nanoparticles have the potential as good markers for nanobiosensor construction and thus they are helpful in disease diagnosis. Although different nanodiagnostic methods have been useful in early, precise and on-site detection of plant diseases caused by various plant pathogens but more research is needed for increasing their specificity, cost-reduction and portability to make them more useful and within reach of farmers. Efforts are also needed for development of necessary skills in public agricultural research organizations for nanotechnological research and increasing public acceptability for nanotechnological applications.

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