

**Advancements in Nanoparticle-Enabled Approaches
for Sustainable Plant Disease Management and
Genetic Enhancement**

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**CENTRE FOR BIOMEDICAL ENGINEERING
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by

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Submitted

*in fulfilment of the requirements of the degree of Doctor of Philosophy
to the*



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*Dedicated to Mummy, Papa and
My Sisters*

Certificate

This is to certify that the thesis entitled '**Advancements in Nanoparticle-Enabled Approaches for Sustainable Plant Disease Management and Genetic Enhancement**' being submitted by **Ms. Anju** to the Indian Institute of Technology Delhi for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. **Ms. Anju** has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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Anju

Abstract

Sustainable plant disease management has long been a major issue in agriculture since the excessive reliance on broad-spectrum pesticides exacerbates chemical resistance, presenting environmental and health hazards. Taking cues from nature's intricate defense mechanisms, scientists are exploiting bioactive agents involved in plant-pathogen/pest interactions to develop novel strategies to combat diseases. Embracing biomolecules in agriculture offers an eco-friendly alternative to chemical pesticides. However, traditional delivery methods for biomolecules often suffer from low utilization rates and low field stability, diminishing the overall effectiveness of active compounds. The motivation for this work comes from the challenges faced in hard-to-transfect animal cells, a similar difficulty that exists in plants due to their rigid cell walls, highlighting the potential to apply successful strategies across diverse systems.

The advent of nanotechnology has facilitated the design of novel delivery systems for biomolecular cargoes, further enhancing their capacity to adhere to the plant surfaces and make disease control strategies effective. Tailored depending upon the extent of infection and type of plant species, innovative nanoparticle strategies maximize the effectiveness of delivery by modifying the size, surface characteristics, and adhesion capacity of the particles to suit particular requirements. The goal of this thesis is to explore how bioactive agents, including genetic and biochemical agents, can serve as alternatives to chemical pesticides, as well as the potential of various nanocarriers for the delivery of biomolecules in plant disease management and genetic enhancement.

In conclusion, by harnessing biomolecules as potent agents, we are effectively leveraging nature's own defense mechanisms to combat pathogens. The integration of nanotechnological advances in biopesticide delivery marks a significant stride in plant disease management. This innovative approach enhances the efficacy of biopesticides while also reducing their environmental impact. This not only ensures targeted delivery but also reduces the need for frequent applications, thereby promoting sustainable agriculture practices. Furthermore, formulations tested in plant disease management often lack *in vitro* cytotoxicity studies to assess their biosafety for human health. In this work, we also conducted these crucial studies to ensure that the formulations are safe for human use, bridging the gap between agricultural and biomedical safety.

Chapter 1 discusses the various challenges and recent developments in the field of plant disease management. In **Chapter 2**, investigates the use of silica nanoparticles with size variations to enhance the uptake efficiency and translocation of AC2-derived double-stranded RNA (dsRNA) for protection against tomato leaf curl New Delhi virus (ToLCNDV). This chapter also highlights how nanoparticles can contribute to the stability of biomolecules, offering a protective shield against environmental degradation and improving their overall effectiveness. However, in actual field conditions, agrochemicals often suffer from low utilization efficiency due to challenges such as poor penetration through plant cell wall and cuticle surfaces.

Thus, **Chapter 3** addresses these challenges by focusing on enhancing the adhesion and retention of biomolecules on tomato plant surfaces, enhancing the utilization efficiency of biomolecules, thus providing protection against *Botrytis cinerea* infections.

While the previous chapter investigated the use of antimicrobial agents for the protection against pathogens, this chapter shifts towards utilizing plant secondary metabolites that do not have direct antimicrobial properties. Instead, these metabolites serve to prime the plant's immune system, triggering an active defense response. By activating the plant's intrinsic immune mechanisms, this approach enables the plant to fight off pathogens more effectively, leveraging its own natural defenses. This strategy holds potential for sustainable, eco-friendly crop protection. Also, dicots have relatively permeable cuticles, monocots have more hydrophobic surfaces, posing unique challenges for biomolecule delivery. Thus, **Chapter 4** investigates by developing smart nanocomposites loaded with secondary metabolites designed for enhanced deposition on superhydrophobic rice leaves, providing protection against *Xanthomonas oryzae* pv. *oryzae* infections.

While the strategies explored in previous chapters significantly improved the delivery, stability, and efficacy of biomolecules in plant disease management, they also underscore the limitations of relying solely on exogenous treatments to address plant health. Ultimately, ensuring long-term advances in plant disease management requires a more integrated approach, particularly through genetic manipulation. **Chapter 5** investigates the use of piezoelectric nanomaterials to facilitate efficient, genotype-independent plant cell transfection via sonoporation. **Chapter 6** discusses the conclusion and future outlook of the thesis.

सारांश

स्थायी पादप रोग प्रबंधन लंबे समय से कृषि में एक प्रमुख मुद्दा रहा है क्योंकि व्यापक स्पेक्ट्रम कीटनाशकों पर अत्यधिक निर्भरता रासायनिक प्रतिरोध को बढ़ाती है, जिससे पर्यावरण और स्वास्थ्य को खतरा होता है। प्रकृति के जटिल रक्षा तंत्र से संकेत लेते हुए, वैज्ञानिक रोगों से निपटने के लिए नवीन रणनीतियों को विकसित करने के लिए संयंत्र-रोगजनक/कीट इंटरैक्शन में शामिल जैवसक्रिय (बायोएक्टिव) एजेंटों का उपयोग कर रहे हैं। कृषि में जैव अणुओं (बायोमोलेक्यूलस) को अपनाने से रासायनिक कीटनाशकों का पर्यावरण-अनुकूल विकल्प मिलता है। हालांकि, जैव अणुओं के लिए पारंपरिक वितरण विधियां अक्सर कम उपयोग दरों और निम्न क्षेत्र स्थिरता से पीड़ित होती हैं, जो सक्रिय यौगिकों की समय प्रभावशीलता को कम करती हैं। इस कार्य की प्रेरणा हार्ड-टू-ट्रांसफेक्ट पशु कोशिकाओं में आने वाली चुनौतियों से आती है, एक ऐसी ही कठिनाई जो पौधों में उनकी कठोर कोशिका भित्ति के कारण होती है, जो विविध प्रणालियों में सफल रणनीतियों को लागू करने की क्षमता को उजागर करती है।

नैनो प्रौद्योगिकी के आगमन से जैव-आणविक कार्गो के लिए नवीन वितरण प्रणालियों के डिजाइन की सुविधा मिली है, जिससे पौधों की सतहों पर चिपकने की उनकी क्षमता में और वृद्धि हुई है तथा रोग नियंत्रण रणनीतियों को प्रभावी बनाया गया है। संक्रमण की सीमा और पौधों की प्रजातियों के प्रकार के आधार पर तैयार की गई नवीन नैनोकण रणनीतियाँ विशेष आवश्यकताओं के अनुरूप कणों के आकार, सतह विशेषताओं और आसंजन क्षमता को संशोधित करके वितरण की प्रभावशीलता को अधिकतम करती हैं। इस शोध प्रबंध का लक्ष्य यह है कि किस प्रकार जैवसक्रिय एजेंट, जिनमें आनुवंशिक और जैवरासायनिक एजेंट शामिल हैं, रासायनिक कीटनाशकों के विकल्प के रूप में काम कर सकते हैं, साथ ही पौधों की बीमारी के प्रबंधन और आनुवंशिक वृद्धि में जैवअणुओं के वितरण के लिए विभिन्न नैनोवाहकों की क्षमता का भी पता लगाना है।

निष्कर्ष रूप में, जैव-अणुओं को शक्तिशाली एजेंट के रूप में उपयोग में लाकर, हम रोगजनकों से लड़ने के लिए प्रकृति की अपनी रक्षा प्रणाली का प्रभावी ढंग से लाभ उठा रहे हैं। जैव कीटनाशक वितरण में नैनो प्रौद्योगिकी की उन्नति का समन्वय, पौध रोग प्रबंधन में एक महत्वपूर्ण प्रगति

है। यह अभिनव दृष्टिकोण जैव कीटनाशकों की प्रभावकारिता को बढ़ाता है और साथ ही उनके पर्यावरणीय प्रभाव को भी कम करता है। यह न केवल लक्षित वितरण सुनिश्चित करता है, बल्कि लगातार अनुप्रयोगों की आवश्यकता को भी कम करता है, जिससे स्थायी कृषि पद्धतियों को बढ़ावा मिलता है। इसके अलावा, पादप रोग प्रबंधन में परीक्षण किए गए फॉर्मूलेशन में अक्सर मानव स्वास्थ्य के लिए उनकी जैव सुरक्षा का आकलन करने के लिए इन विट्रो साइटोटॉक्सिसिटी अध्ययनों का अभाव होता है। इस थीसिस में, हमने यह सुनिश्चित करने के लिए इन महत्वपूर्ण अध्ययनों का संचालन भी किया कि कृषि और बायोमैडिकल सुरक्षा के बीच के अंतर को कम करते हुए, मानव उपयोग के लिए फॉर्मूलेशन सुरक्षित हैं।

अध्याय 1 पौधों की बीमारी प्रबंधन के क्षेत्र में विभिन्न चुनौतियों और नवीन विकास पर चर्चा करता है। अध्याय 2 टमाटर लीफ कर्ल न्यू दिल्ली वायरस (ToLCNDV) के खिलाफ संरक्षण के लिए AC2-व्युत्पन्न डबल-स्ट्रैंडेड RNA (dsRNA) के अवशोषण दक्षता और स्थानांतरण को बढ़ाने के लिए विभिन्न आकारों के सिलिका नैनोकणों के उपयोग की जांच करता है। यह अध्याय यह भी उजागर करता है कि नैनोकण जैव-अणुओं की स्थिरता में कैसे योगदान दे सकते हैं, पर्यावरणीय क्षरण के खिलाफ एक सुरक्षात्मक ढाल प्रदान करते हैं और उनकी समग्र प्रभावशीलता में सुधार करते हैं। हालांकि, वास्तविक क्षेत्र स्थितियों में, कृषि रसायनों की उपयोगिता दक्षता अक्सर कम होती है, क्योंकि पौधों की कोशिका भित्ति और क्यूटिकल सतहों में उनका प्रवेश ठीक से नहीं हो पाता।

इसलिए, अध्याय 3 इन चुनौतियों को टमाटर पौधों की सतहों पर जैव-अणुओं के आसंजन और अवधारण को बढ़ाने पर ध्यान केंद्रित करके संबोधित करता है, जिससे जैव-अणुओं की उपयोग दक्षता में वृद्धि होती है, और इस प्रकार बोटीटिस सिनेरिया संक्रमण के खिलाफ संरक्षण प्रदान करता है।

जबकि पिछले अध्याय ने रोगजनकों के खिलाफ संरक्षण के लिए रोगाणुरोधी एजेंटों के उपयोग की जांच की थी, यह अध्याय पौधों के द्वितीयक चयापचयों (मेटाबोलाइट्स) के उपयोग की ओर ध्यान देता है, जिनमें प्रत्यक्ष रोगाणुरोधी गुण नहीं होते। इसके बजाय, ये चयापचय पौधे की प्रतिरक्षा

प्रणाली को सक्रिय करते हैं, जिससे एक सक्रिय रक्षा प्रतिक्रिया उत्पन्न होती है। पौधे की आंतरिक प्रतिरक्षा तंत्र को सक्रिय करके, यह दृष्टिकोण पौधे को रोगजनकों से अधिक प्रभावी ढंग से लड़ने में सक्षम बनाता है, जो इसकी प्राकृतिक रक्षा का लाभ उठाता है। यह रणनीति टिकाऊ, पर्यावरण-अनुकूल फसल संरक्षण के लिए संभावनाएं रखती है। साथ ही, डाइकॉट्स में अपेक्षाकृत पारगम्य क्यूटिकल्स होते हैं, जबकि मोनोकॉट्स में अधिक हाइड्रोफोबिक सतहें होती हैं, जो जैव-अणु वितरण के लिए अद्वितीय चुनौतियां प्रस्तुत करती हैं। इसलिए, अध्याय 4 सुपरहाइड्रोफोबिक चावल की पत्तियों पर बेहतर जमाव के लिए डिज़ाइन किए गए द्वितीयक चयापचयों से लदे स्मार्ट नैनोकम्पोजिट्स के विकास की जांच करता है, जो जैथोमोनास ओराइजे pv. ओराइजे संक्रमण के खिलाफ संरक्षण प्रदान करता है।

जबकि पिछले अध्यायों में खोजी गई रणनीतियों ने पौधों की बीमारी प्रबंधन में जैव-अणुओं के वितरण, स्थिरता और प्रभावशीलता में काफी सुधार किया, वे केवल बाहरी उपचारों पर निर्भर रहने की सीमाओं को भी रेखांकित करते हैं। अंततः, पौधों की बीमारी प्रबंधन में दीर्घकालिक प्रगति सुनिश्चित करने के लिए एक अधिक एकीकृत दृष्टिकोण की आवश्यकता है, विशेष रूप से आनुवंशिक हेरफेर के माध्यम से। अध्याय 5 सोनोपोरेशन के माध्यम से कुशल, जीनोटाइप-स्वतंत्र पौधे कोशिका ट्रांसफेक्शन की सुविधा के लिए पीज़ोइलेक्ट्रिक नैनोमटेरियल्स के उपयोग की जांच करता है। अध्याय 6 थीसिस के निष्कर्ष और भविष्य के दृष्टिकोण पर चर्चा करता है।

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List of Abbreviations

°C	Degrees Celsius
µg	Microgram
µl	Microliter
mg	Miligram
NPs	Nanoparticles
HAMPS	Herbivore associated molecular patterns
DAMPs	Damage-associated signals
PRRs	Pattern recognition receptors
ROS	Reactive oxygen species
ETI	Effector-triggered immunity
PTI	Pattern Triggered Immunity
SA	Salicylic acid
dsRNAs	Double stranded RNAs
GM	Genetically modified
RNAi	RNA interference
SAR	Systemic acquired resistance
ToLCNDV	tomato leaf curl New Delhi virus
ENPs	Engineered nanoparticles
MSNPs	Mesoporous silica nanoparticles
SNPs	Silica nanoparticles
CTAB	N-cetyltrimethylammonium bromide
TEOS	Tetraethylorthosilicate
APTES	3-aminopropyltriethoxysilane
DEA	Diethanol amine
TEA	Triethanol amine
<i>N. b.</i>	<i>Nicotiana benthamiana</i>
FITC	Fluorescein isothiocyanate
FESEM	Field Emission scanning electron microscopy
HRTEM	High Resolution Transmission electron microscopy
SAXS	Small Angle X-ray Scattering

LAXRD	Low angle X ray Diffraction
HAXRD	High angle X ray Diffraction
FTIR	Fourier transform infrared
SEM	Scanning Electron Microscopy
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
DMEM	Dulbecco's Modified Eagle Medium
PI	Propidium Iodide
DMSO	Dimethyl sulphoxide
PFA	Paraformaldehyde
DAB	3,3'-diaminobenzidine
NBT	Nitro blue tetrazolium
FCP	Folin-Ciocalteu's phenol
TA	Tannic acid
PEC	Polyelectrolyte complex
FAO	Food and Agricultural Organisation
BLB	Bacterial leaf blight
Mel	Melatonin
BTNPS	Barium titanate nanoparticles
pDNA	Plasmid DNA
GFP	Green fluorescent Protein
GUS	β-glucuronidase
kDa	Kilodalton
Calcein AM	Calcein acetoxymethyl ester
PBS	Phosphate Buffered Saline
mM	Millimolar
mm	Millimeter
nM	Nanomolar
G.E.	Genetic engineering
rpm	Rotations per minute