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Extracellular Vesicles: A New Green Technology for Sustainable Farming in India

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Abstract

Indian agriculture faces challenges like soil degradation, biodiversity loss, pesticide resistance, and climate-induced stresses. Conventional farming has increased yields but caused ecological harm. Plant-derived extracellular vesicles (PDEVs) are natural nanovesicles containing proteins, RNAs, lipids, and metabolites that offer an eco-friendly approach to sustainable farming. This review summarises recent advances in the discovery, biogenesis, isolation, and characterisation of PDEVs, focusing on their roles in plant growth, defence, stress tolerance, and microbial interactions. Studies from India and globally show that PDEVs facilitate intercellular communication, enhance defence by delivering small RNAs to silence pathogen genes, and recruit beneficial microbes. Field trials demonstrate their effectiveness as natural biopesticides, reducing fungal and insect damage, and as biofertilizers, improving nutrient uptake and crop yields by 15–20%. PDEV treatments also increase resilience to drought, salinity, and heat, supporting climate-smart agriculture. Challenges remain in scalable production, stability under field conditions, and regulatory approval. PDEVs represent a significant advance in green biotechnology for Indian farming, offering multifunctional benefits to reduce chemical inputs and promote resilient food systems.

Keywords: Plant-derived extracellular vesicles (PDEVs); Biofertilizers; Biopesticides; Green biotechnology; RNA interference (RNAi).

Introduction

Indian agriculture faces extraordinary challenges such as soil degradation, increasing pest resistance, climate-induced droughts, and heat stress. This threatens the livelihoods of millions and the nation's food security. For decades, reliance on chemical fertilisers, pesticides, and monoculture farming has driven productivity but has also led to environmental

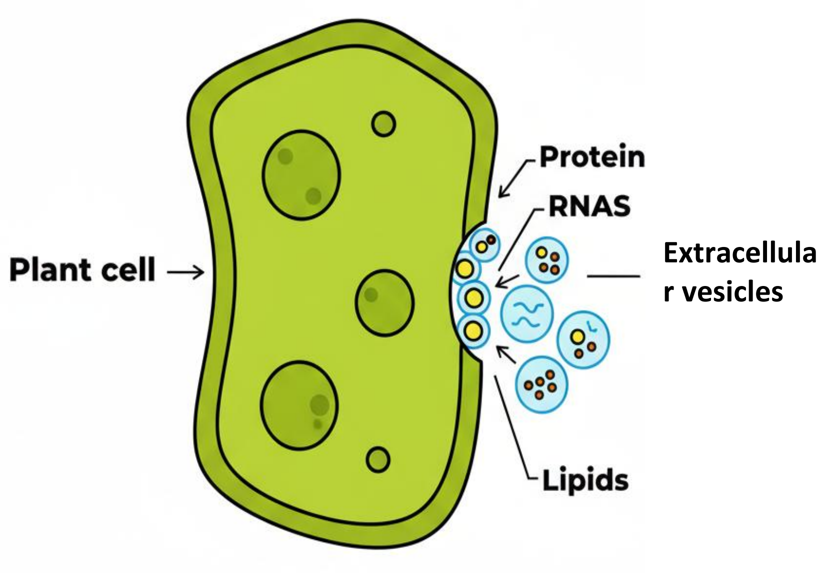

Figure 1: Plant cells naturally release EVs containing bioactive molecules like proteins, RNAs, and metabolites

pollution, loss of biodiversity, and declining soil health. In recent years, innovations in plant biology and nanotechnology have promised a paradigm shift: plant-derived extracellular vesicles (PDEVs). These tiny, naturally occurring, membrane-bound particles are secreted by plant cells and contain diverse bioactive molecules (Fig.1). They are emerging as eco-friendly alternatives that could revolutionise agriculture, reduce harmful chemical use, and promote sustainable farming practices (ICAR, 2023).

This article explores the science of PDEVs, their mechanisms of action, recent advances, applications in Indian agriculture, and the challenges facing their large-scale development and deployment.

Historical Background and Discovery of Plant-Derived EVs

The concept of extracellular vesicles originated from mammalian cell biology, where they are recognised as mediators in immune responses, development, and disease (Raposo & Stoorvogel, 2013). However, their recognition in plants is relatively recent, first identified in the late 2000s with the advent of microscope techniques, proteomics, and high-throughput sequencing (Regente et al., 2017).

Researchers observed vesicles in plant apoplastic fluids, root exudates, and edible plant tissues, suggesting their role in cell signalling and defence (Rutter & Innes, 2017). These vesicles, now called plant extracellular vesicles (PEVs), exhibit diverse sizes, compositions, and functions, from immune modulation to nutrient transport (Mu et al., 2021).

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The discovery that plant EVs can carry small RNAs (sRNAs) capable of silencing pathogen genes (Cai et al., 2014) ignited interest in their potential as natural biopesticides, promoting sustainable crop protection strategies.

Biogenesis and Types of Plant-Derived Extracellular Vesicles

Plant-derived extracellular vesicles (EVs) are lipid bilayer-enclosed particles released by plant cells into the extracellular space. Their biogenesis occurs through several pathways that contribute to the diversity of vesicle types and functions observed in plants.

Classical Biogenesis Pathways

A primary route for plant EV formation involves multivesicular bodies (MVBs), specialised intracellular compartments where inward budding of the endosomal membrane generates intraluminal vesicles. These vesicles are released as exosome-like particles upon fusion of MVBs with the plasma membrane, typically measuring 30 to 150 nanometers. This process, conserved in many eukaryotes, is orchestrated by a multi-protein machinery known as ESCRT (endosomal sorting complex required for transport), which sorts and packages cargoes destined for secretion (Valadi et al., 2007; Lian et al., 2022).

Another fundamental mechanism is plasma membrane blebbing, in which microvesicles form by outward budding directly from the cell surface. These microvesicles tend to be larger (100 to 1000 nanometers) and are enriched in signalling molecules that mediate local and systemic communication between plant cells (Regente et al., 2017).

Noncanonical Secretion Routes

Plant cells use specialised pathways such as exocyst-positive organelles (EXPOs), unique double-membrane structures that secrete vesicles containing proteins and RNAs, often in stress response. EXPO-mediated secretion expands the plant's capability to deliver extracellular messages during critical physiological events (Wang et al., 2021).

Autophagy-related secretion represents another unconventional pathway, where autophagosomes, instead of fusing with degradative vacuoles, merge with the plasma membrane to release defensive EVs. This mechanism allows plants to deploy defence molecules efficiently during stress or pathogen attack (Liang et al., 2022).

Passage Through the Plant Cell Wall

A unique challenge for plant EVs is their release through the rigid plant cell wall, a dense extracellular matrix composed of fibres like cellulose and lignin. To overcome this barrier, plant EVs commonly carry degrading enzymes such as pectinases and cellulases, which temporarily loosen the cell wall network, permitting vesicular transit (Regente et al.,

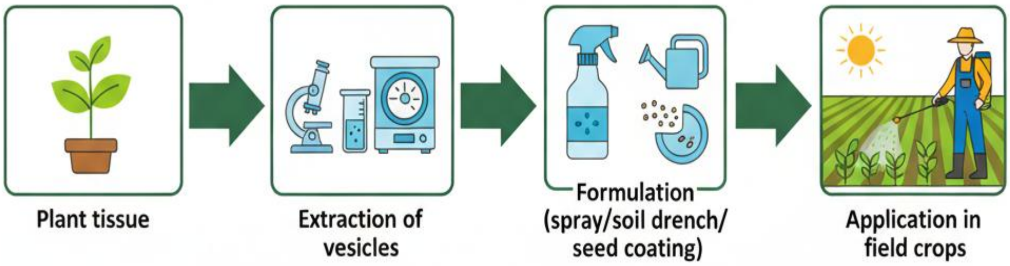

Figure 2: Journey of EVs from Plant to Field Application through sprays, coatings, or soil drenching

2017). This enzymatic remodelling might be tightly controlled to balance cell wall integrity with the need for vesicle secretion.

Isolation and Characterisation of Plant-Derived Extracellular Vesicles

Plant EVs can be isolated from diverse sources, including leaves, roots, seeds, fruits, edible plant juices (e.g., ginger, carrot), and cell culture systems like callus and protoplast suspensions (Lian et al., 2022). Extraction involves differential ultracentrifugation for debris removal and concentration of vesicles, followed by purification methods such as density gradient centrifugation to improve purity, or size exclusion chromatography to sort vesicle subpopulations. Rapid precipitation kits are also used for edible EVs, albeit with some trade-off in purity (Mu et al., 2021).

Standard characterisation includes transmission electron microscopy (TEM) for vesicle morphology, nanoparticle tracking analysis (NTA) for size and concentration, proteomics and lipidomics for cargo composition, and RNA sequencing to identify messenger and small RNAs (Fig.2) (Liang et al., 2022).

Molecular Composition and Biological Functions

Plant extracellular vesicles encapsulate complex cargos that include proteins as defence enzymes and signalling factors. Lipids that constitute their membranes (phosphatidylcholine, sphingolipids), and nucleic acids, particularly small RNAs capable of regulating gene expression in both plants and interacting organisms (Lian et al., 2022; Cai et al., 2014). Additionally, EVs carry secondary metabolites like flavonoids and phenolics, contributing antimicrobial and antioxidant properties.

Functionally, plant EVs mediate intercellular communication to coordinate development and responses to environmental cues. They play critical roles in defence by transporting small RNAs that silence pathogenic genes, thereby inhibiting fungal, bacterial, or viral infections

(Wang et al., 2021). Furthermore, they modulate beneficial microbial interactions by recruiting symbiotic rhizobacteria and deterring pathogens. Lastly, EVs prime plants against abiotic stresses as drought, heat, and salinity, by activating stress response pathways (Liang et al., 2022).

Roles in Plant Physiology and Defence

- Intercellular Communication: Coordinating growth, development, and systemic response signalling.
- Defence Mechanisms: Transferring small RNAs that silence pathogenic genes in fungi, bacteria, or viruses (Cai et al., 2014; Wang et al., 2021).
- Microbial Symbiosis: Recruiting beneficial root microbiota and deterring pathogens.
- Stress Tolerance: Priming plants for drought, salinity, and thermal stress by activating defence pathways (Liang et al., 2022).

Recent Advances and Emerging Applications

Biofertilizers

Recent studies highlight the application of PDEVs (Fig.3) as natural biofertilizers capable of enhancing nutrient uptake and soil fertility. PDEVs from beneficial rhizobacteria like *Rhizobium* and *Bacillus* facilitate nitrogen fixation, phosphate solubilization, and enhance root development, offering yield improvements of 15–20% in field crops (ICAR,

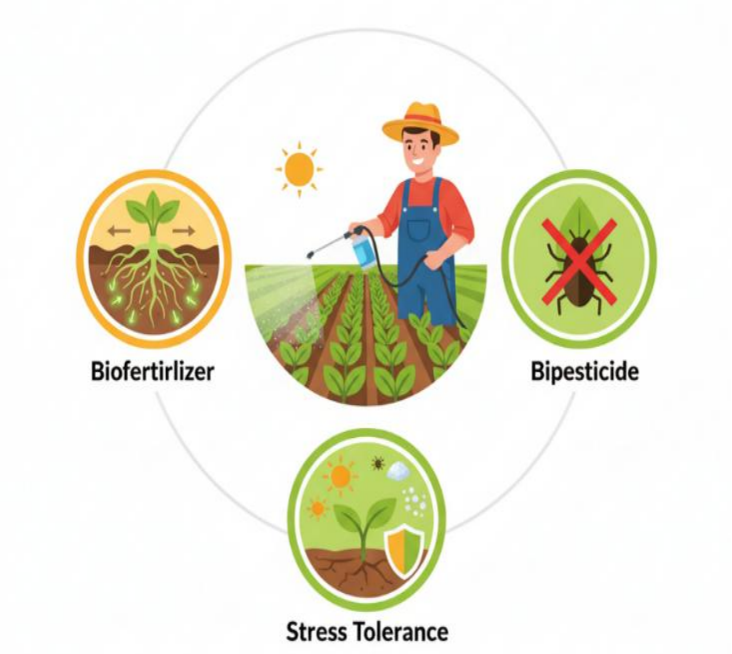

Figure 3: Applications of EVs in Agriculture
EVs act as natural biofertilizers, biopesticides, and stress protectors in crops

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2023; Zhu et al., 2025). These biological inputs provide an eco-friendly alternative to synthetic fertilisers, contributing to soil health and reducing environmental pollution (Jo et al., 2025).

Biopesticides

The delivery of RNA interference molecules via PDEVs offers species-specific pest and pathogen control (Fig.4). Recent field and laboratory data demonstrate that PDEV sprays loaded with small RNAs effectively silence essential genes in fungal pathogens like *Fusarium* and insect pests such as *Helicoverpa armigera*, decreasing disease severity and larval survival by up to 70% and 60%, respectively (Gupta et al., 2022; Koch et al., 2018). Such targeted biopesticides decrease chemical pesticide usage and circumvent resistance development.

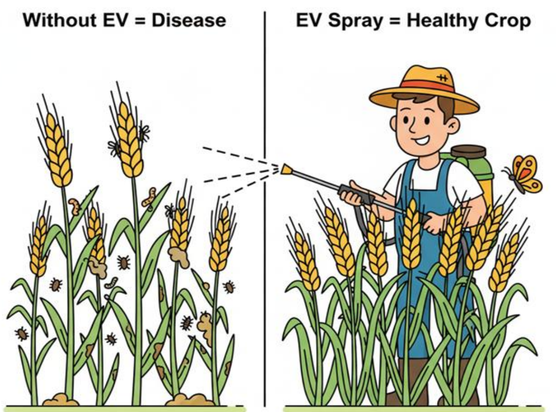

Figure 4: Case Study – EV Sprays in Wheat or Cotton

Stress Resilience

PDEVs have emerged as novel agents for bolstering plant tolerance to abiotic stressors, including drought, salinity, and heat. Their treatments elicit activation of antioxidant enzymes, osmoprotectants, and stress signalling pathways, reducing oxidative damage and maintaining physiological functioning under stress (Liang et al., 2022; Zhu et al., 2025). This capability is vital for climate-resilient agriculture in India's increasingly variable environments.

Medical and Nutraceutical Uses

Beyond agriculture, PDEVs are proving valuable in medical sciences and nutrition. Their natural composition and nanosize confer excellent biocompatibility, making them attractive drug carriers for anti-inflammatory, anticancer, and antioxidative therapeutics (Yang et al., 2023; Jo et al., 2025). Edible PDEVs from ginger, citrus, and other plants also modulate gut microbiota, promoting intestinal health and offering potential as functional foods and nutraceuticals (Yang et al., 2023).

Notably, innovative vaccine delivery methods employing PDEVs have been explored. Loading mRNA vaccines into PDEVs derived from citrus juice has shown enhanced stability and effective oral delivery potential, marking a milestone in non-invasive immunisation strategies (Zhu et al., 2025).

Challenges and large-scale applications:

- Scalable production: Efficient, low-cost methods to isolate large quantities of PDEVs from crop tissues.
- Stability: Maintaining EV integrity under field conditions (temperature, UV exposure).
- Cargo loading: Controlling the content of EVs for targeted effects.
- Regulatory pathways: Establishing safety guidelines for EV-based products. Institute-led initiatives, including the Indian Society for Extracellular Vesicles (InSEV), are actively working to standardise protocols and conduct regulatory assessments (Fig.5) (InSEV, 2025).

Future Perspectives

PDEVs are at the forefront of a paradigm shift in sustainable agriculture. Future directions include:

- Genetic manipulation of plants to overproduce beneficial EVs.
- Engineering EVs to carry specific bioactives for targeted pest or stress management.
- Employing nanotechnology to enhance EV stability and delivery.
- Integrating EVs as part of integrated pest and nutrient management practices compatible with organic farming.

Advancements in bioinformatics and systems biology will facilitate understanding cargo selection, vesicle trafficking, and cross-kingdom communication mechanisms, accelerating translation from lab to field (Lian et al., 2022).

Conclusion

Plant-derived extracellular vesicles represent an innovative, eco-friendly, and versatile tool for promoting sustainable farming practices in India. Their capacity to carry natural bioactives, induce plant resistance, and target pests with precision offers a tangible pathway toward reducing chemical dependency. While further research and regulatory frameworks are

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needed, PDEVs hold the promise to usher in a new green revolution—fostering resilient crops, healthier ecosystems, and sustainable livelihoods for Indian farmers.

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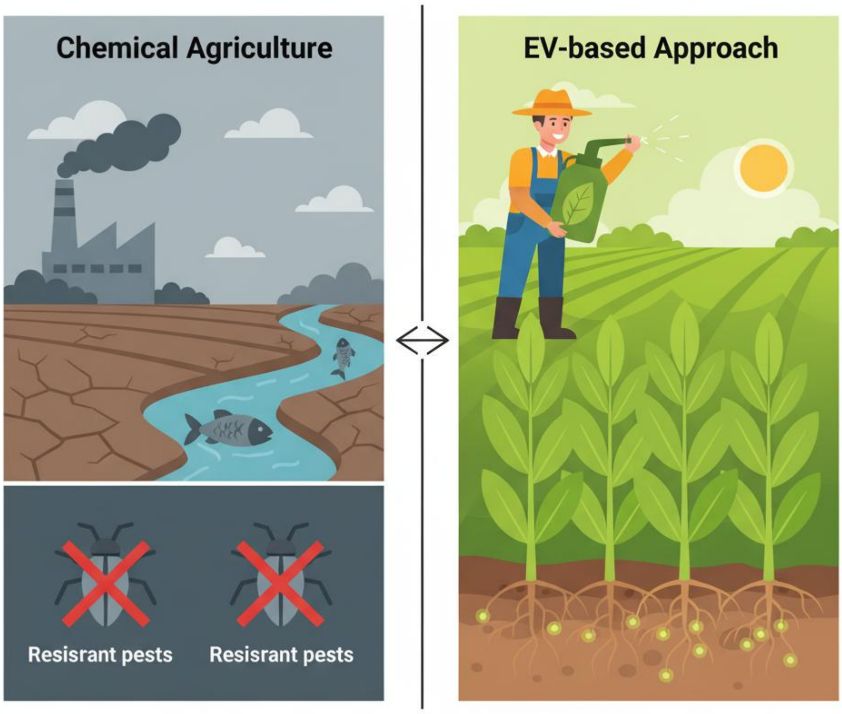

Figure 5: EVs vs Chemicals (Comparison Infographic)
Comparison of chemical-based farming vs. EV-based farming practices.

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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