



REVIEW ARTICLE

Sustainable encapsulation of bio-active agents and microorganisms in electrospun nanofibers: A comprehensive review

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Abstract

Nanotechnology is a technological discipline focused on the design, fabrication and utilization of structures, systems and devices through the manipulation of atoms and molecules at the nanoscale. A significant advancement in this field is the development of a nanocarrier system for microbe encapsulation using electrospun nanofibers. These nanofibers, characterized by their diameters in the nanometric range, are produced through nanotechnology. The electrospinning technique is a versatile method that fabricates these nanofibers from polymer solutions, including polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO), polyethylene glycol (PEG) and chitosan, using high voltage. Nanofibers play a crucial role in various fields, including environmental remediation, medicine, agriculture and textiles. Beneficial microorganisms are microbial cells that aid crops by protecting them from pathogens, supplying essential nutrients and alleviating both biotic and abiotic stresses. Several techniques have been developed to encapsulate microorganisms within nanofibers, with electrospinning being the most widely applied method. This technique effectively traps microbial cells while preserving their viability for extended periods without causing harm. Microorganisms such as bacteria, fungi and viruses, as well as fertilizers, pesticides and growth hormones, can be successfully encapsulated within nanofibers. This review provides a comprehensive overview of nanofibers, including their characterization, the polymers utilized [such as chitosan, PVA, PEO, PEG and alginate] and the electrospinning process with its variations. It also discusses techniques for encapsulating microbial cells within nanofibers and their current applications in agriculture.

Keywords

cell viability; electrospinning; encapsulation; microbes; nanofiber

Introduction

In a world with a growing population, large-scale food production is essential for the survival of humankind. Crops play a significant role in food production, with factors such as soil fertility and soil microbiota being crucial. Fertilizers are vital for maintaining and improving soil fertility and crop productivity (1). However, traditional fertilizers are not fully accessible to plants due to their insoluble forms in the soil. As a result, beneficial soil microorganisms have been increasingly

utilized as alternatives to synthetic fertilizers in recent decades (2, 3). These microorganisms positively influence plants either directly or indirectly by enhancing nutrient uptake, producing siderophore and facilitating nitrogen fixation (4).

One of the most effective methods to deliver these beneficial microorganisms to the soil is by applying seeds coated with plant growth-promoting microbes. These microbes colonize plant roots and support crop development (5). However, bacteria are vulnerable to physical damage and various environmental stresses, such as fluctuations in temperature, humidity and pressure, which can destabilize their cellular content and harm the cells. Such damage can lead to a loss of microbial cell viability, thereby reducing their beneficial effects on crop plants (6).

To address these challenges, encapsulation has emerged as an effective technology that extends the shelf life of microbial cells, protects them and allows for gradual release. Encapsulation also facilitates the handling of microbial cells (7). This bio formulation maintains microbial activities linked to plant growth promotion, guaranteeing optimal cell viability and survival while enhancing the rhizosphere colonization of microbial cells in plant roots (8).

Carriers used in microbial cell encapsulation serve as protective barriers against environmental stresses and enable storage under dry conditions. Various polymers are utilized to form nanofibers, including PEO, PVP, poly(diethoxy) phosphazene (PPZ), chitosan and PVA. Among these, PVA is particularly favored due to its excellent physical and chemical properties (9). These polymers act as carriers for cell entrapment through several methods, including freeze-drying, extrusion, spray-drying and emulsification. However, these methods have notable drawbacks, such as high fabrication costs, reduced microbial survival rates, uncertainty regarding root colonization, limitations in encapsulating multiple strains simultaneously and challenges in scaling up to industrial levels (10).

Nanotechnology has facilitated the production of electrospun nanofibers, which can serve as nanocarriers for encapsulating microbial cultures. The electrospinning technique is a versatile method that converts concentrated polymeric solutions into nanofibers under high voltage in a controlled environment. This technique is a promising platform for encapsulation systems, as these fibers can entrap nanoparticles, enzymes, proteins and entire cells (11). This encapsulation method allows microbial cells to be immobilized within seeds for extended periods without losing viability, a process known as immobilization.

The term "co-mobilization" refers to the simultaneous entrapment of multiple microbial strains within a seed (3). Co-mobilization provides plants with a mixture of microbes that offer essential nutrients, phytohormones and protection from both biotic and abiotic stresses, thereby supporting growth and development. Moreover, seeds encapsulated with exopolysaccharide (EPS)- producing microbes can help protect plants from abiotic stresses such as drought and salinity. By encapsulating phosphorus-solubilizing bacteria, potash-releasing bacteria and nitrogen-fixing bacteria in nanofibers, crop plants can access vital nutrients, potentially reducing the need for chemical fertilizers.

New combinations of polymeric nanofibers are expected to play a crucial role in the development of nanofibers for agricultural applications. This review aims to analyze current nano formulation techniques, focusing on nanofiber development and the chemicals used for fabrication. Additionally, it provides a brief overview of the electrospinning technique and its variations, with a primary emphasis on the agricultural application of these technologies.

Choice of polymeric carriers

Carrier materials are essential in influencing the quality and efficacy of formulations. An optimal carrier must be biodegradable, non-toxic and harmless to humans, the environment and microbial cells. It must also preserve microbial cultures and sustain their efficacy over an extended duration. Furthermore, it must be economical and manageable. Moreover, it should allow the gradual release of microorganisms. It should also facilitate the incorporation of nutrients or supplements, be compatible with beneficial microbes and exhibit physicochemical qualities that enable high water retention capacity (9). Fig. 1 illustrates the polymers used in electrospinning for the synthesis of nanofibers, encompassing both natural and synthetic types. Polymers are classified as either natural or synthetic based on their origin. Selected polymeric carriers and their characteristics are discussed in the following sections.

Chitosan: Chitosan, which consists of N-acetyl glucosamine units (GlcNAc), can be utilized as a carrier for plant-beneficial microorganisms (12). Chitosan possesses several desirable properties, including biodegradability, non-toxicity and ease of use. It promotes the synthesis of osmo-regulators in plants and has notable antimicrobial activities (13). However, its limited mechanical resistance and low chain flexibility are significant drawbacks that impede the effective entrapment of microbial cells (14).

Combining chitosan with other polymers, such as alginate and PVA, can enhance its properties by improving its structure and physicochemical stability (15). Recent studies have shown that a mixture of chitosan and 2% alginate is effective for entrapment of *Methylobacterium oryzae*. This combined formulation yielded better results in establishing tomato seedlings compared to the use of alginate alone (12).

Sodium alginate: Sodium alginate is a polysaccharide that is abundantly found in the cell walls of brown algae, *Azotobacter* and *Pseudomonas* (16). This polymer comprises β -D-mannuronate and α -L-guluronate residues, which contain carboxylate groups that carry a negative charge. It serves as a substrate for the entrapment of plant growth-promoting microorganisms due to its biodegradability, biocompatibility, low cost, non-toxicity and ability to withstand acidic soil conditions. Furthermore, it can accommodate a large number of microbial cells and facilitates their slow and gradual diffusion (17).

Polyethylene oxide: Polyethylene oxide is a neutral, biocompatible, non-toxic and hydrophilic polymer that is widely used in tissue engineering and drug delivery. It is not absorbed in the gastrointestinal tract, exhibits low immunogenicity and demonstrates good compatibility with live cells. Polyethylene glycol, another polymer derived from ethylene oxide, differs primarily in its molecular weight. The

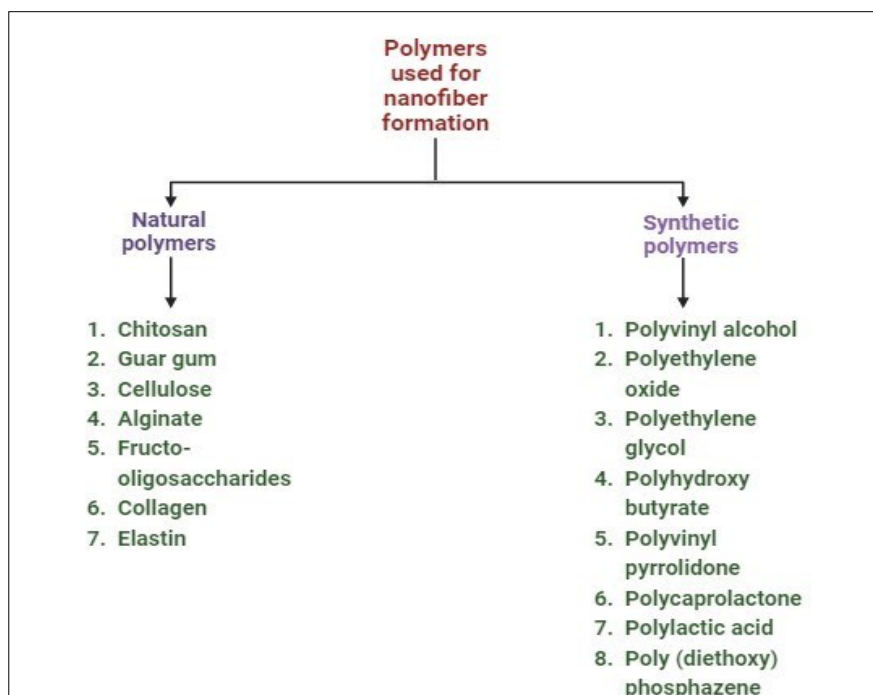


Fig. 1. Polymers in electrospinning process for fabrication in the development of microbial nanocarrier.

combination of PEO with both synthetic and natural polymers enhance the physical and mechanical properties of the resulting materials (18).

Polyvinyl alcohol: Polyvinyl alcohol is a synthetic, hydrophilic, biodegradable polymer used in the synthesis of nanofibers. It is semi-crystalline, biocompatible and exhibits high thermal and chemical stability (19). Increasing the concentration of PVA raises its viscosity, leading to greater chain entanglement, which helps overcome surface tension and results in the formation of beadless nanofibers. Additionally, PVA has the potential to enhance water uptake and maintain higher moisture content around seeds.

Nanofiber: Nanofibers are fibers produced through nanotechnology, characterized by diameters in the nanometric range (1 nm to 1000 nm) (20) and are most commonly fabricated using the electrospinning technique (21). These fibers are classified as one-dimensional nanomaterials with diameters ranging from tens to hundreds of nanometers. Nanofibers can be constructed from a wide variety of materials, including natural polymers such as chitosan and cellulose, synthetic polymers like PVA, polylactic acid and polycaprolactone, as well as hybrid polymers, such as mixtures of chitosan and PVA. Natural polymers typically offer better biocompatibility and lower immunogenicity, while synthetic polymers provide greater flexibility in production and modification (22).

These fibers are utilized for the encapsulation or entrapment of various biological entities, including mammalian cells (23), bacterial strains (24), spores (25), yeast cells (26), nanoparticles, antibiotics and plasmids (27). In agriculture, nanofibers are utilized in seed treatment with synthetic and biological components to promote seedling emergence and inhibit pathogen infections. They possess special properties, such as high mechanical and thermal stability, high porosity, tunable release rates and a high surface-to-volume ratio. Additional features like biocompatibility, uniform size distribution and biodegradability make nanofibers highly

effective in addressing environmental issues such as waste water treatment and pollutant removal from soils (28, 29).

One of the primary uses of nanofibers in agriculture is seed coating, which facilitates the sustained delivery of agricultural inputs such as nutrients and phytohormones to improve seed germination and crop growth (30, 31). Nanofiber-based membrane filters have been employed in irrigation systems to remove heavy metals from water (32). Furthermore, they are utilized to detect pesticide levels in water (33) and to protect plants from pathogens by encapsulating fungicides. Beyond agriculture, nanofibers find applications in various fields, including healthcare, energy storage and environmental remediation (34).

Encapsulation and its techniques

As mentioned earlier, encapsulation is the process of inoculating microbial cultures into a chosen carrier. This process involves three distinct steps, which are illustrated in Fig. 2.

- In the first step, microorganisms or active ingredients are mixed and absorbed into a polymeric matrix.
- The second step is a mechanical process in which a liquid solution is dispersed under mixing conditions to form solid particles.
- In the final step, the particles produced during the second step undergo polymerization and physicochemical stabilization (3).

Encapsulation can be classified into macro, micro and nano-encapsulation based on the size of the particles produced by different methods. Several techniques are available for encapsulating microbes within the polymer mixture, which will be discussed in this manuscript.

Ionic gelation: Ionic gelation is a widely used method due to its low production cost and accessible processing conditions (35). In this technique, a sodium alginate aqueous solution containing microbial cells is dispersed in a divalent cation

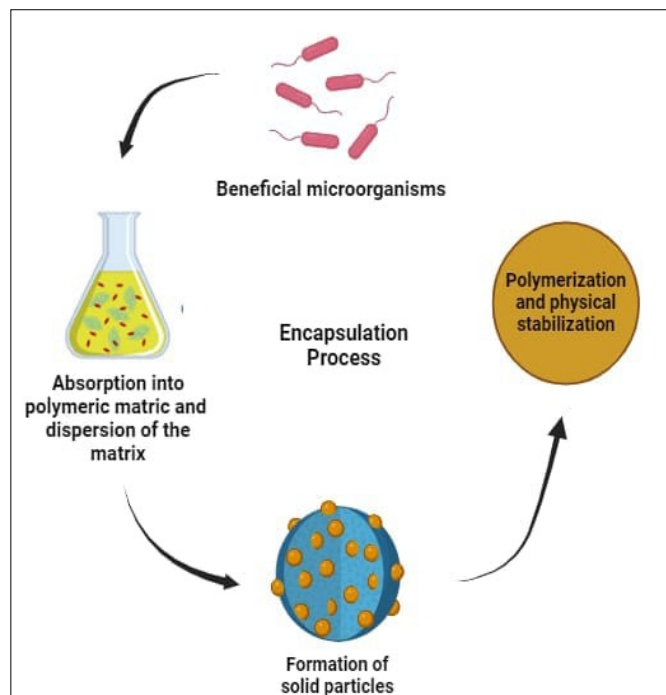


Fig. 2. Encapsulation process of microbial cells.

solution of calcium chloride. Upon solidification of the droplets, a hydrogel is generated via the interaction between negatively charged sodium alginate polymer chains and Ca^{2+} (calcium cations) (36). This technique generates homogenous beads of diverse dimensions, from nanometers to millimeters, depending upon the needle size; even nanosized beads can be manufactured utilizing specialist encapsulating apparatus.

Alternate polymers, such as pectinate derivatives and guar gum, can be used instead of sodium alginate and Ba^{2+} (barium (II) cation) and Cu^{2+} (cupric ion) can be substituted for calcium chloride (37).

Spray drying: The spray drying technique is a well-defined and straight forward dehydration process used for synthesizing immobilized microbial inoculum (38). In this method, bacteria are dispersed in the carrier and the final emulsion is introduced into a drying chamber where atomization occurs with the addition of hot air and gas. As the liquid evaporates, it creates ventilation, leaving behind a dry powder or microcapsule (39).

Emulsification: In the emulsification method, surfactants and drugs create either an oil-in-water or water-in-oil emulsion. Polymers are dispersed in water, which is immiscible with oils and the bacterial solution is then added while mixing (36). Commonly used polymers in this technique include sodium alginate, while oils such as soybean oil and gelatin are often employed. Table 1 presents additional techniques for encapsulating microbial cultures in nanofibers along with their applications.

Other techniques

Solvent evaporation: The solvent evaporation encapsulation technique is widely used to create microspheres or microcapsules containing active ingredients, such as drugs, biomolecules, or other substances. This process involves forming a polymer-based shell around the active ingredients, which provides protection and controls their release (52).

Table 1. Encapsulation techniques of microbial cells and their applications for sustainable agricultural development

Encapsulation method	Microorganisms	Carrier	Host	Purpose	Reference
Extrusion	<i>Kosakonia radicincitans</i>	Amidated pectin	Radish	Desalination and osmo-protectant	(40)
	<i>Bacillus subtilis</i>	Sodium alginate	Lettuce	Plant growth promotion	(41)
	<i>Pantoea agglomerans</i>	Sodium alginate	Rice	Desalination	(42)
	<i>Klebsiella oxytoca</i> + <i>B. subtilis</i>	Sodium alginate	Cotton	Biocontrol of <i>Rhizoctonia solani</i> under saline conditions	(43)
	<i>Methylobacterium oryzae</i>	Sodium alginate + chitosan	Tomato	Seed germination and plant growth promotion	(12)
	<i>Azospirillum brasilense</i>	Sodium alginate	Wheat	Plant growth promotion	(44)
Spray drying	<i>Streptomyces fulvissimus</i>	Chitosan + gellan gum	Wheat	Biocontrol of <i>Gaeumannomyces graminis</i>	(39)
	<i>Bacillus megaterium</i>	Chitosan + maltodextrin		Bioremediation of salinized soils	(45)
Emulsification	<i>Pseudomonas putida</i>	Sodium alginate + paraffin	Thale cress	Plant growth promotion	(46)
	<i>Pseudomonas fluorescens</i>	Sodium alginate + soybean oil	Potato	Biocontrol of <i>Fusarium solani</i>	(47)
	<i>Sinorhizobium meliloti</i>	Canola oil + xanthan gum	Alfalfa	Nodulation and plant growth promotion	(48)
Electrospinning	<i>P. agglomerans</i> + <i>Burkholderia caribensis</i>	Polyvinyl alcohol	Soy- bean	Plant growth promotion	(49)
	<i>Methylobacterium aminovorans</i>	Polyvinyl alcohol	Ground nut	Improving germination and growth of plant	(50)
	<i>Trichoderma viride</i> spores	Polyethylene oxide/poly-acrylamide/chitosan		Plant protection	(25)
Coacervation	<i>Bacillus thuringiensis</i>	Sodium alginate + chitosan		Insect pest management (<i>Spodoptera litura</i>)	(51)
	<i>B. subtilis</i> and <i>P. fluorescens</i>	Chitosan + sodium alginate		Promoting plant growth and suppressing soil borne pathogens	(36)
	<i>Rhizobium</i> sp.	Gelatin + arabic gum	Legume crops	Efficiency in nitrogen fixation and promoting plant growth	(5)

Coacervation: Coacervation is a micro encapsulation technique based on phase separation, commonly employed for encapsulating bioactive compounds, drugs, or other substances. The method involves depositing a polymer around an active core material by forming polymer-rich droplets, or coacervates, which solidify into protective capsules. This technique is widely utilized in the pharmaceutical, cosmetic and food industries (53). Coacervation is classified into two primary categories. Simple coacervation utilizes a single polymer system, whereas complex coacervation entails the interaction of two or more oppositely charged polymers, often a protein and a polysaccharide.

Fluidized bed coating: Fluidized bed coating is a technique commonly used in the pharmaceutical, food and chemical industries for applying thin, uniform coatings to solid particles such as powders, granules, or tablets. This process suspends solid particles in a fluidized state by passing a stream of air or gas through them while a coating solution or molten material is applied. This technique is particularly valuable for coating particles with polymers to achieve controlled release, protection, or taste masking (54).

Nanofiber formation by electrospinning technique

Various methods are used to model nanofibers, including template synthesis, the drawing process, phase separation, carbon dioxide laser supersonic drawing (CLSD) and electrospinning (55). Among these, electrospinning, also known as electrostatic spinning, is a nanoscale technique widely recognized for its effectiveness (56).

Electrospinning is an eco-friendly, simple and cost-effective process for producing nanofibers from different polymer solutions by applying a high-voltage electric field (57). During this process, a droplet forms at the exit of the spinneret. When a high electric field is applied, with an electrode submerged in the polymer solution and a counter-electrode positioned away from the spinneret, the resulting Maxwell stress stretches the droplet, creating a Taylor cone and initiating a jet. The high-voltage field shapes the droplet and as field strength increases, electric charges accumulate

on the outer layer, causing electrostatic repulsion at the polymer tip. This leads to the formation of an elongated droplet, known as the "Taylor cone." The electric charges stretch and elongate the polymer solution, which then settles on the collector as polymer fibers (58). Through this stretching and subsequent solvent evaporation, nanoscale fibers are formed (59). Fibers made from various polymers demonstrate excellent mechanical properties and high surface areas, which make them ideal for numerous applications.

The basic electrospinning setup includes a high-voltage source, a syringe, a metal needle and a metal collector, as depicted in Fig. 3 (60). The figure illustrates microbial cells in nanofiber formation, applied across fields such as pharmaceuticals, agriculture and environmental protection, along with the electrospinning setup itself. Alternative types of electrospinning techniques include wet electrospinning, coaxial electrospinning and melt electrospinning. Melt electrospinning, for instance, is particularly useful in applications that avoid toxic solvents, low-solubility polymers and high-viscosity solutions.

One key advantage of electrospinning is its ability to synthesize ultra-fine fibers with nanometer-range diameters, providing maximum surface area and enhanced mechanical properties (61). Electrospinning techniques are categorized into six types, as shown in Fig. 4 (62) and is illustrated in Table 2.

Electrospinning of bacteria, fungi and viruses

Bacteria: Electrospun nanofibers have been widely used to encapsulate bacteria, primarily from the probiotic group. Probiotics are live microbial cells that provide health benefits to the host when introduced into the body. However, their low viability and high sensitivity to environmental stress present challenges for practical applications. To address this, a study proposed the development of a new probiotic delivery system (68). To maintain the viability and efficient delivery of probiotics, various electrospun nanofibers, including PVA nanofiber, PVA/chitosan hybrid nanofiber, PVP nanofiber and PVP/chitosan nanofiber, have been employed for probiotic encapsulation or entanglement (19).

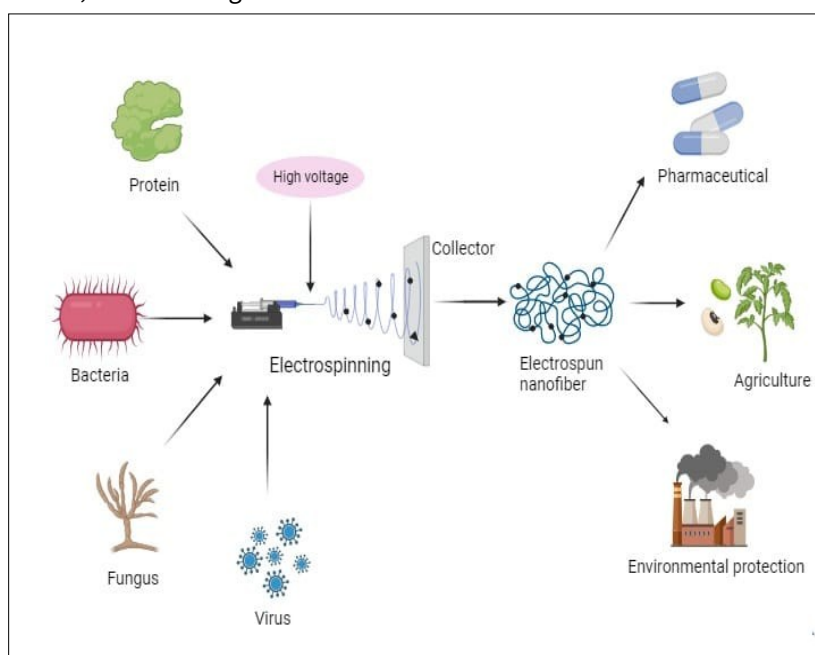


Fig. 3. A schematic process of horizontal electrospinning set up and the application of electrospun nanofibers.

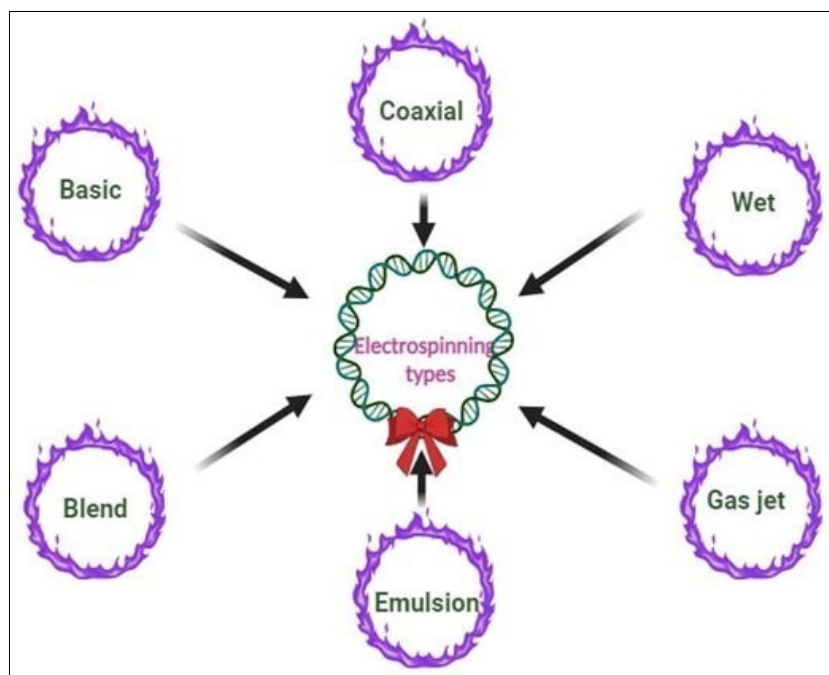


Fig. 4. The given picture depicts the electrospinning types.

Table 2. Different categories of electrospinning techniques

Name of the technique	Description	Reference
Basic electrospinning	Involves a single polymer. Blend Electrospinning: Combines polymers, adjusting their ratios to enhance drug delivery applications by allowing for tunable, gradual drug release.	(63)
Blend electrospinning	In blend electrospinning, polymers in different phases (water or oil) are blended together using a stabilizer. The solution is then electrospun by applying high voltage electricity to the solution and a collector. The solution extrudes from a nozzle, forming a jet that dries and deposits fibers on the collector.	(63)
Coaxial electrospinning	Begins drug release with an initial burst from the sheath, followed by sustained release from the core.	(64)
Melt electrospinning	Uses polymer melts instead of polymer solutions, resulting in slower, moderate drug release compared to solution-based fibers, which often exhibit an initial burst phase.	(65)
Gas jet electrospinning	Integrates the electrospinning unit with a gas jet device, enclosing the spinning fluid capillary with a gas jet, leading to finer, more uniform fibers.	(66)
Emulsification method	Forms core-shell-structured nanofibers through an oil-in-water or water-in-oil emulsion of drugs and surfactants. This method uses a single nozzle, protecting encapsulated materials from external environmental conditions.	(67)
Wet electrospinning	Wet electrospinning is a technique for creating nanofibrous materials with a controlled 3D structure. It uses a liquid collector instead of a metal collector.	

In addition to probiotics, non-probiotic bacteria have also been incorporated into nanofibers for applications in therapeutics, biosensors, biocatalysis and agriculture. *Lactobacillus*, a commonly used probiotic bacterium, is frequently entrapped in these nanofibers due to its health benefits and compatibility with nanofiber-based delivery systems.

Electrospun nanofiber systems offer significant applications in agriculture, including seed inoculation, stress management and nanofiber-coated fertilizers and pesticides. Rhizobacteria are beneficial microbes that colonize plant roots and enhance crop resilience by protecting plants from biotic and abiotic stresses while promoting nutrient uptake. However, maintaining their viability during seed treatments and storage remains a challenge. To address this, these bacteria are encapsulated in nanofibers such as PVA, chitosan, PEG and PEO, which help improve their survival within seeds compared to unencapsulated forms. For instance, the bacterium *P. agglomerans* was shown to improve germination rates, leaf number, leaf length, root dry weight and shoot length in agricultural applications (49).

A biosensor is a device that measures biological and chemical reactions by generating a signal proportional to the analyte concentration within a reaction. It is used to detect and identify specific components within cells or tissues. *Gluconobacter oxydans* as a biosensor for glucose detection was reported in a study (69). The immobilization of *Gluconobacter oxydans* into poly (ϵ -caprolactone) (PCL) nanofibers enhanced oxygen and glucose transfer to the cells, resulting in a faster sensor response time and lower glucose detection.

Cyanobacteria are photosynthetic organisms rich in fatty acids, minerals and vitamins, commonly used as food supplements. *Spirulina*, a well-known genus of cyanobacteria, is frequently processed-dried and milled-before being encapsulated into nanofibers for various applications, including spinal cord injury treatment, tissue engineering and antibacterial therapy. It was also proposed in a study that *Spirulina* biomass incorporated into PEO nanofibers could function as an extracellular matrix for stem cell culture and spinal cord injury treatment (70). *Spirulina* is valued for its ability to produce high-quality biopolymers ideal for nanofiber fabrication. For instance,

biopolymers like polyhydroxybutyrate produced by *Spirulina sp.* LEB 18 contains cyanobacterial phenolic compounds used in nanofiber formation, which exhibit antifungal, antioxidant and antibacterial properties. These synthesized nanofibers were found to inhibit the growth of *Staphylococcus aureus* ATCC 25923, supporting their potential use in food packaging applications (71).

Fungi: Research on fungi encapsulated in nanofibers has primarily targeted their biocatalytic properties rather than their biotherapeutic potential. Since water-soluble nanofibers are unsuitable for applications in aqueous environments like waste water, coaxial electrospun nanofibers, featuring a water-miscible core with a hydrophobic shell, are commonly used. Certain fungi, such as *Candida tropicalis*, demonstrate extensive pollutant-degradation capabilities, making them valuable for waste water purification, despite being pathogenic to humans. In a study, the fungus was successfully incorporated into nanofibers with a PVP core and a polyvinylidene fluoride-hexafluoropropylene shell through coaxial electrospinning (26). Once encapsulated, *C. tropicalis* effectively degraded phenols, fermented ethanol in olive mill waste water and showed toxicity against *Escherichia coli*.

Other fungi, including *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, are also effective for water purification. When these heat-inactivated fungi were encapsulated in PVA and cellulose acetate hybrid nanofibers, they facilitated aflatoxin B2 removal from contaminated water by binding the toxin to their surfaces, resulting in water that was less cytotoxic to human fibroblasts (72).

Virus: Viruses, while pathogenic organisms, can offer therapeutic or medicinal benefits when administered to specific tissues at appropriate doses. Encapsulating viruses in nanofibers helps maintain their viability, making them valuable tools in combating bacteria, targeting cancer and enabling gene delivery. Table 3 outlines the entrapment of microbial cultures within electrospun nanofibers, detailing their applications alongside specific polymers.

The vaccinia virus exhibits biocidal properties against colorectal cancer. Nonetheless, direct injection into people poses complications owing to its immunogenicity, potentially resulting in detrimental health effects. In a study, it was highlighted that incorporating the vaccinia virus into poly

(lactic-co-glycolic acid) (PLGA) electrospun nanofibers offers an effective strategy for controlled virus release, significantly enhancing colon cancer cell death (73).

Viability of microbial cells after encapsulation

The sustainability of microbial cultures after encapsulation in nanofibers was evaluated by assessing the colony-forming abilities of the cells on agar plates. There were slight changes in cell viability following entanglement with nanofibers. A study reported that exposing *Staphylococcus albus*, *E. coli* and bacteriophages to PVA nanofibers at room temperature resulted in a complete loss of viability, as evidenced by a reduction in their colony-forming ability after one month (74). In contrast, samples stored at 4°C exhibited some loss of viability after three months but remained completely stable at -20°C and -55°C. The sustainability of *P. agglomerans* ISIB55 and *B. caribensis* ISIB40 in spinning solution and nanofibers was determined using the plate dilution method on yeast mannitol agar (49). The sustainability of both strains was measured in terms of log10 colony-forming units per gram.

According to a study, polymers are excellent carriers for encapsulation bacteria, thereby supporting bacterial sustainability (75). The introduction of *Azotobacter chroococcum* into the spinning solution did not affect its sustainability. After encapsulating *Azotobacter chroococcum* in nanofibers, cultures stored at 4°C for six months exhibited a gradual reduction in cell numbers, while those kept at ambient temperature experienced a significant decrease in sustainability due to the unstable conditions surrounding the nanofibers (76).

A study further examined the sustainability of *M. aminovorans* cells entrapped in PVA nanofibers (50). When stored at room temperature, the cell numbers decreased over time. The authors noted that the viability of *M. aminovorans* cells could be preserved for up to 30 days at optimal temperatures when encapsulated in nanofibers, thanks to the protective polymeric matrix that shields against microbial culture dehydration. The sustainability of *B. subtilis* in polymeric nanofibers was analyzed using the spread plate technique (57). The viability of bacteria was monitored monthly for up to six months at room temperature, revealing a loss of viability by the sixth month due to heat transfer from the external environment through the nanofibers.

Table 3. Incorporation of microbial cells into electrospun nanofibers and their uses in agriculture

Microorganisms	Nanofiber polymer	Purpose	Reference
Bacteria			
<i>P. agglomerans</i>	Polyvinyl alcohol	Seed coating	(49)
<i>Gloconobacter oxydans</i>	Polycaprolactone	Biosensor for glucose	(69)
<i>P. fluorescens</i>	Sodium alginate + soybean oil	Biocontrol of <i>F. solani</i>	(47)
<i>A. brasiliense</i>	Sodium alginate	Plant growth promotion	(44)
<i>B. thuringiensis</i>	Sodium alginate + chitosan	Insect pest management (<i>S. litura</i>)	(51)
<i>M. aminovorans</i>	Polyvinyl alcohol	Improving germination and growth of plant	(50)
<i>Rhizobium sp.</i>	Gelatin + arabic gum	Efficiency in nitrogen fixation and promoting plant growth	(5)
<i>S. meliloti</i>	Canola oil + xanthan gum	Nodulation and plant growth promotion	(41)
<i>Spirulina sp.</i>	Poly hydroxybutyrate	Food packaging	(71)
Fungi			
<i>T. viride</i>	Polyethylene oxide, chitosan	Inhibit the growth of phytopathogenic strains such as <i>Fusarium</i> and <i>Alternaria</i>	(25)

The sustainability of a microbial consortium (*B. subtilis* and *Serratia marcescens*) encapsulated in nanofibers was noted to decline when stored at room temperature (77). Polyvinyl alcohol/chitosan nanofibers protected the microorganisms from external environmental conditions (57). The encapsulation of *A. brasilense* and *P. fluorescens* in a polymer mixture-maintained cell viability at 10^9 CFU/g and 10^8 CFU/g, respectively, after 12 months of storage at optimal temperatures (78). Similarly, the sustainability of *Lactobacillus acidophilus* (79), *Bradyrhizobium japonicum* (11) and *P. agglomerans* (49), when entrapped in PVA nanofibers, was maintained for extended periods under ambient temperatures storage, as reported by the authors.

A study concluded that nanofibers are the best carriers for encapsulating microorganisms, effectively maintaining the sustainability of microbial cultures (57). The polymers used in nanofiber fabrication protect cellular integrity when exposed to external environmental conditions. The average load of *B. subtilis* cells suspended in the polymeric spinning solution was 10^{16} CFUs. After the spinning process, $14.06 \log_{10}$ CFUs were encapsulated in the nanofibers, with some loss of microbial cells due to mechanical stress and pressure caused by solvent evaporation during the application of high voltage to the bacterial-polymer mixture.

The immobilization of *Lactobacillus acidophilus* in PVA and PVP nanofibers could extend the viability of the bacterium for up to 85-90 days (79). The sustainability of this culture increased to 78.6-90% when encapsulated in agro-waste-based nanofibers. The viability of *T. viride* spores was preserved when encapsulated in nanofibers composed of PEO, polyacrylamide and chitosan (25). These encapsulated fungal spores could inhibit the growth of phytopathogenic strains such as *Fusarium* and *Alternaria*. Rice rhizobacteria *Paenibacillus* IBGE-MAB1, immobilized in nanofibers for seed coating and vigor germination, demonstrated a higher survival rate of 98% after immobilization in nanofibers (80).

Applications of electrospinning nanofiber in agriculture

The applications of electrospun nanofibers in agriculture encompass seed coating (81), plant protection through the

entanglement of fungicides (82) and the entrapment of pesticides (83), herbicides (84) and agrochemicals such as fertilizers (85) and phytohormones (86). These nanofibers also enhance plant yield by encapsulating microbial cells. Fig. 5 illustrates the application of electrospun nanofibers in agriculture. In this field, nanofibers are utilized for pest and disease management, nutrient management and for addressing moisture and temperature stress, as well as for salinity tolerance. This is achieved through the encapsulation of beneficial microbes and the use of specific polymers, which will be explained in detail in the following section.

Plant protection: A valuable method for delivering active ingredients in soybean seeds involves seed coating using electrospinning and cellulose diacetate (CDA) polymer (31). In another study, CDA was selected due to its solubility in solvents and its suitability for electrospinning (87). In one of the study, two active ingredients, abamectin and fluopyram, were encapsulated separately into the nanofibers (31). Abamectin is an anthelmintic that demonstrates strong activity against certain arthropods and nematodes while exhibiting minimal toxicity to non-target organisms. Fluopyram, a succinate dehydrogenase inhibitor fungicide, has also proven effective as a nematocide (88, 89).

One of the studies evaluated the ability of fluopyram-loaded nanofibers to reduce the mycelial growth of the fungal pathogen *Alternaria lineariae* through a 16-day *in vitro* fungal assay. The results indicated that samples containing fluopyram-loaded nanofibers exhibited smaller mycelial growth compared to the control samples. Even after 16 days, fluopyram-loaded nanofibers consistently inhibited fungal growth, with a mycelial diameter of 3.5 cm for the fungicide-containing nanofibers versus 7.5 cm for the controls (88, 89).

Preliminary bioavailability experiments demonstrated the effectiveness of abamectin-loaded nanofibers in immobilizing nematodes using a roundworm (*Caenorhabditis elegans*)-based bioassay (88, 89). These findings showed that abamectin-loaded nanofibers were more effective than the control samples. Fig. 6 illustrates the application of nanofibers incorporated with beneficial microbes for protecting crops

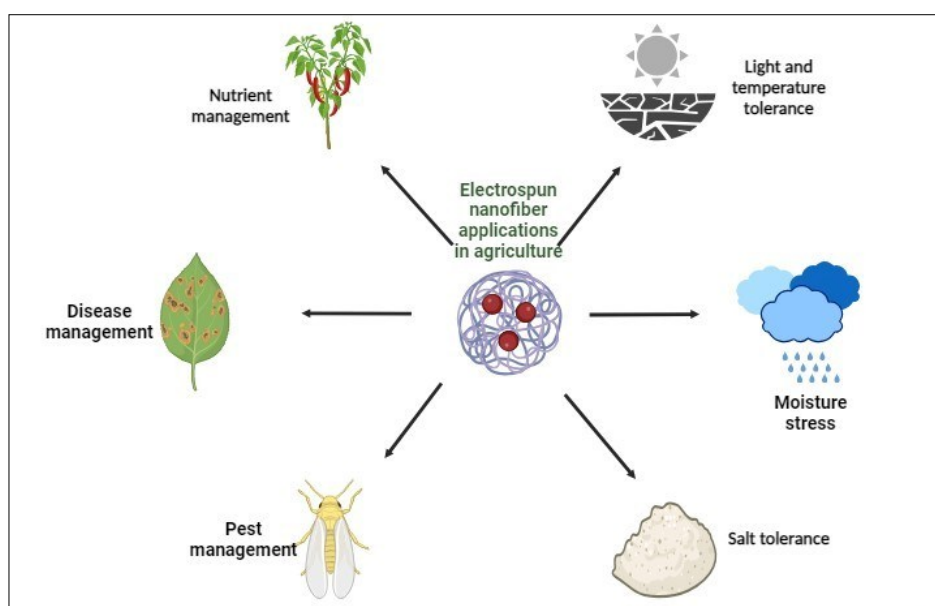


Fig. 5. Electrospun nanofibers application in agriculture.

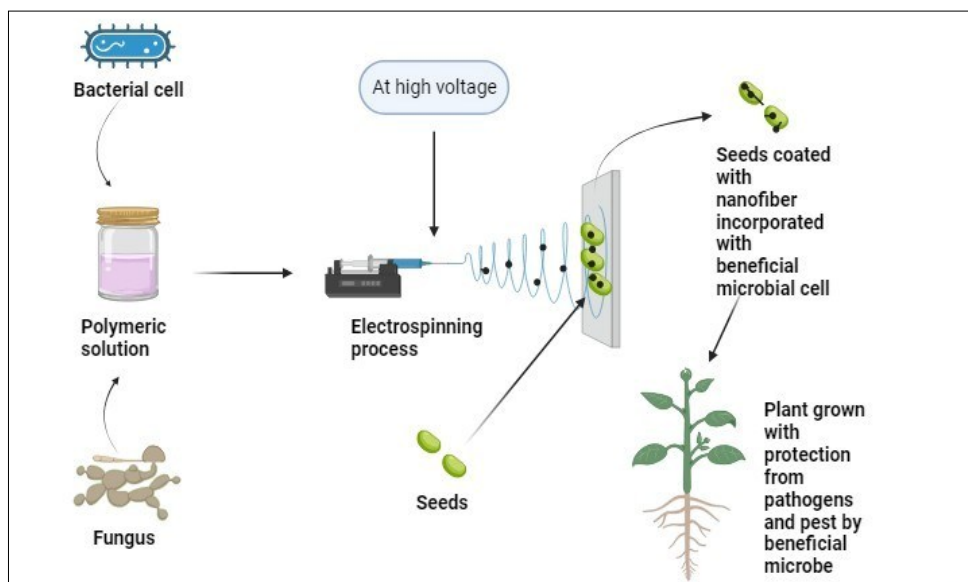


Fig. 6. Nanofibers used in plant protection from pathogens and pests.

from pathogens and pests. The biocontrol agents and beneficial microbes were entangled in the nanofiber through electrospinning and used for seed coating. Upon seed germination, the plants obtained nutrients from the beneficial microbes and protection from pests and diseases through biocontrol agents, thus safeguarding the crops from biotic and abiotic stresses throughout their growth period.

It has been demonstrated that *B. megaterium* PB50 and *B. pumilus* PB18 exhibit antagonistic activity against fungal pathogens (90). Incorporating these microorganisms into nanofibers could protect microbial cells from pathogens. The author reviewed the significance of nanotechnology as a management strategy to address the challenges posed by plant pathogens, emphasizing that nanomaterials are effectively employed to manage and control soil-borne pathogens (91).

Moisture stress: Drought is a significant limiting factor in agriculture, obstructing crop growth, reducing productivity and affecting both morphological and physiological processes (92). Water scarcity presents a key challenge for plant growth, constraining crop yields on arable land (93). Prolonged drought periods and a lack of water can render crops unsustainable. Drought conditions lead to yield losses, contributing to severe food shortages (94). To address this issue, polymer-based seed coatings have been shown to enhance seed tolerance to drought by absorbing and retaining substantial amounts of water. A potassium-based copolymer was utilized in the hydro-absorber coating for cereal seeds such as barley, wheat and rye (95). In addition to the hydrophilic polymer coating, two growth-promoting substances, humic acid and biplantol, were also incorporated into the coating formulation to stimulate germination and root development in the crops. The results indicated that a thicker coating positively influenced germination and promoted earlier seedling growth compared to thinner coatings.

The ZEB polymer (starch-g-poly-(2-propenamide-co-propenoic acid) potassium salt) has been utilized for seed coating and the effects of seeding rates and polymer coatings on the germination and growth of both cold and warm-season grasses have been explored (96). Coated seeds exhibited

improved growth and development under low seeding rates and drought conditions. Similarly, a study found that a PEG 8000 polymer coating on canola seeds mitigated the effects of moisture stress by enhancing germination in laboratory conditions (97).

B. megaterium produces 3-hydroxybutanone (acetoin), a precursor to 2,3-hydroxybutanone, which has been shown to alleviate drought stress in plants. Incorporating this bacterium into nanofibers presents a potential strategy for extended drought stress protection, as the slow release of the bacterium from the nanofibers would ensure sustained plant defence over time (98).

In one of the studies, the author recommended using hydrogels for seed coating, which are three-dimensional structures composed of physically or chemically cross-linked hydrophilic chains (99). Hydrogels can absorb large amounts of water without dissolving and can release the absorbed moisture to plant roots while binding with ions and nutrients for sustained release (100). Incorporating hydrogels into soil enhances water and fertilizer retention while increasing soil aeration (101).

The germination performance of wheat seeds coated with either linear synthetic polyacrylamide or agar-blended natural hydrogel under drought stress was examined in a study (102). The findings revealed that polyacrylamide-coated seeds performed better than control seeds. However, while hydrogel coatings improved seedling growth under drought-simulated conditions compared to both polyacrylamide-coated and control seeds, the study indicated that polyacrylamide-coated seeds performed worse under drought conditions than uncoated seeds. The authors concluded that natural hydrogel coatings exhibit greater potential for promoting seed growth under drought stress compared to linear hydrophilic synthetic polymer coatings. The use of acrylamide copolymer hydrogel for coating wheatgrass (*Agropyron cristatum*) seeds under different watering conditions and soil textures has been investigated (103). The results indicated improved seedling establishment in both scenarios.

Temperature and light tolerance: Temperature-activated

polymers (polyacrylate-based, with an activation temperature of 12 °C) was used to coat maize seeds, protecting them from prolonged exposure to cold soils, where seeds are more susceptible to pathogens (104). The authors observed that polymer-coated seeds exhibited higher emergence rates compared to uncoated seeds when planted in soil with a temperature below 10 °C and maintained under these conditions for more than 20 days before emergence.

Salt tolerance: High salt concentrations in soil and saline water adversely affect crops by impeding seed germination and growth. Elevated levels of sodium (Na^+) and chloride (Cl^-) can induce toxicity in seeds. However, employing seed coating and seed priming technologies during the seedling stage may enhance salt tolerance and boost crop productivity. A study examined the effects of commercially available hydrophilic polymer coatings on the growth of five turfgrass species irrigated with saline water (105). Notably, the seeds coated with the polymer did not exhibit any negative impact on seedling emergence when irrigated with saline water.

The effects of a hydrophilic polymer coating (polyacrylamide) and two plant growth regulators-gibberellic acid and salicylic acid-on the performance of dill plants under saline conditions were examined (106). The hydrophilic polymer coating had a negative effect, leading to the accumulation of Na^+ in the roots and leaves of the dill plants. This resulted in an ion imbalance due to reduced K^+ uptake and suppression of antioxidant enzymes. In contrast, seed treatment with gibberellic acid and salicylic acid effectively reduced the uptake of Na^+ ions by the plants across various saline concentrations.

The effect of chitosan, a natural polymer, on plant seeds was investigated (107). Seeds soaked in chitosan solutions at various concentrations exhibited germination and growth compared to untreated seeds. Additionally, the chitosan coating enhanced seed tolerance to NaCl, promoting germination and seed growth when planted in pots with different NaCl concentrations.

Prospects

The proposed approach holds significant potential for enhancing sustainable agricultural practices by improving plant health, reducing reliance on chemical fertilizers and pesticides and promoting soil health. Future research should concentrate on optimizing the electrospinning process to ensure the viability of beneficial microbes during and after delivery. Key areas for refinement include polymer concentration, solvent selection and electrospinning conditions, all aimed at enhancing the efficiency and stability of microbe encapsulation. Additionally, efforts should focus on encapsulating a wider variety of beneficial microbes within individual nanofibers and exploring combinations of different polymers to create nanofibers with unique properties. Developing biodegradable and biocompatible nanofibers is essential for minimizing the environmental impact of agriculture. Integrating electrospun nanofibers with precision agriculture technologies, such as sensors and drones, could facilitate targeted delivery of microbes, improving efficiency and reducing waste. Research should also address the scalability of electrospinning technology to overcome

challenges related to mass production and cost-effectiveness. Extensive field trials are necessary to assess the effectiveness of electrospun nanofiber delivery systems under diverse environmental conditions and crop types, providing vital data on their practical applications. Collaborative efforts among microbiologists, materials scientists, agricultural engineers and farmers will be crucial for advancing this technology and ensuring its successful integration into agricultural practices.

Conclusion

Nanotechnology is an innovative approach with significant potential for application in bioremediation, medicine, pharmaceuticals and agriculture. In agriculture, it is employed to enhance plant growth, nutrition and protection against adverse environmental conditions and pathogens. This is achieved by utilizing nanoparticles or advanced polymers in the form of electrospun nanofibers within plants and tissues, providing a sustainable solution. These nanofibers can also be immobilized with plant growth-promoting bacterial bioinoculants to reduce the excessive use of fertilizers and pesticides, making this approach more feasible and functional. Given the crucial role of nanotechnology in modern agricultural practices, further research in this field is essential to maximize its benefits in agriculture and related areas.

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Authors' contributions

AKAM and KGS carried out the collection of information, drafting, editing, formatting and analysis of the manuscript. AKAM, KGS and GM participated in the sequence alignment and drafted the manuscript. KK, AKAM and KGS assessed the data provided. PM, AKAM and KGS provided the methodology for writing the manuscript. KJ, AKAM and KGS participated in its design and coordination. AP, AKAM and KGS carried out the reference management of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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Declaration of generative AI and AI-assisted technologies in the writing process

While preparing the manuscript, the authors used Grammarly to improve the language and readability. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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