



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, polyvinyl pyrrolidone, polyethylene oxide, polyethylene glycol 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, polyvinyl alcohol (PVA), polyethylene oxide (PEO), polyethylene glycol (PEG) and alginate) and the electrospinning process with its variations. It also discusses techniques for encapsulating microbial cells within nanofibers and their applications in agriculture in the current context.

Keywords

microbes; encapsulation; electrospinning; nanofiber; cell viability

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 Polyethylene oxide (PEO), polyvinyl pyrrolidone (PVP), poly(diethoxy) phosphazene (PPZ), chitosan and polyvinyl alcohol. Among these, polyvinyl alcohol (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, as suggested by Bashan et al. (9), 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. **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.

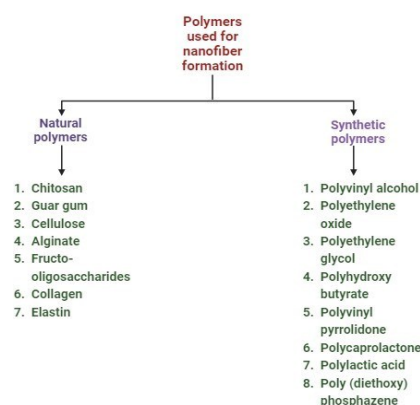


Fig. 1. Polymers in electrospinning process for fabrication in the development of microbial nanocarrier.e

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, as reported by Vassilev et al. (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). The authors suggest that 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 combination of polyethylene oxide 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 (1nm to 1000nm) (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 polyvinyl alcohol, polylactic acid and polycaprolactone, as well as hybrid polymers, such as mixtures of chitosan and polyvinyl alcohol. 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 wastewater 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.

1. In the first step, microorganisms or active ingredients are mixed and absorbed into a polymeric matrix.
2. The second step is a mechanical process in which a liquid solution is dispersed under mixing conditions to form solid particles.
3. 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.

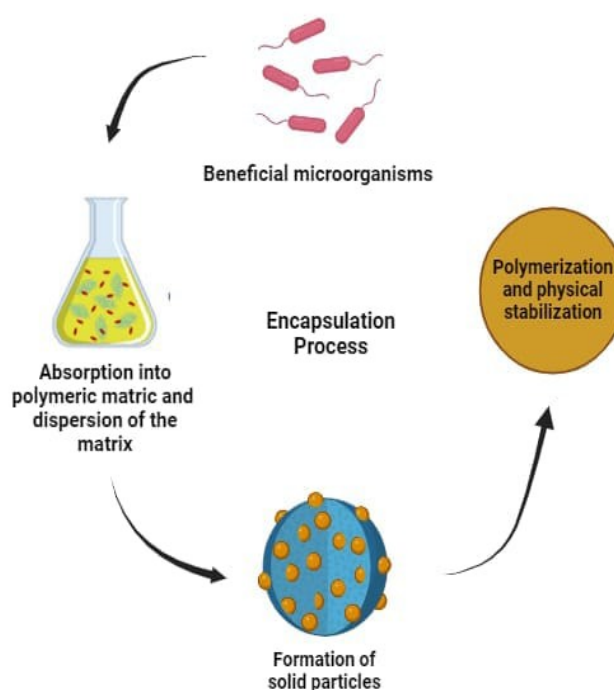


Fig. 2. Encapsulation process of microbial cells.

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 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+} 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+} and Cu^{2+} can be substituted for calcium chloride (37).

Spray drying

The spray drying technique is a well-defined and straightforward 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 (42).

Coacervation

Coacervation is a microencapsulation 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 (43). 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.

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	(100)
	<i>Bacillus subtilis</i>	Sodium alginate	Lettuce	Plant growth promotion	(101)
	<i>Pantoea agglomerans</i>	Sodium alginate	Rice	Desalination	(102)
	<i>Klebsiella oxytoca</i> + <i>Bacillus subtilis</i>	Sodium alginate	Cotton	Biocontrol of <i>Rhizoctonia solani</i> under saline conditions	(103)
	<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	(104)
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	(105)
Emulsification	<i>Pseudomonas putida</i>	Sodium alginate + paraffin	Thale cress	Plant growth promotion	(41)
	<i>Pseudomonas fluorescens</i>	Sodium alginate + soybean oil	Potato	Biocontrol of <i>Fusarium solani</i>	(40)
	<i>Sinorhizobium meliloti</i>	Canola oil + xanthan gum	Alfalfa	Nodulation and plant growth promotion	(106)
Electrospinning	<i>Pantoea agglomerans</i> + <i>Burkholderia caribensis</i>	Polyvinyl alcohol	Soy- bean	Plant growth promotion	(59)
	<i>Methylobacterium aminovorans</i>	Polyvinyl alcohol	Ground nut	Improving germination and growth of plant	(68)
	<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>)	(107)
	<i>Bacillus subtilis</i> and <i>Pseudomonas fluorescens</i>	Chitosan + Sodium alginate		Promoting plant growth and suppressing soil borne pathogens	(36)
	<i>Rhizobium sp.</i>	Gelatin + Arabic gum	Legume crops	Efficiency in nitrogen fixation and promoting plant growth	(5)

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 (44).

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 (45). Among these, electrospinning, also known as electrostatic spinning, is a nanoscale technique widely recognized for its effectiveness (46).

Electrospinning is an eco-friendly, simple and cost-effective process for producing nanofibers from different polymer solutions by applying a high-voltage electric field (47). 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 (48). Through this stretching and subsequent solvent evaporation, nanoscale fibers are formed (49). 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 (50). The figure illustrates microbial cells in nanofiber formation, applied across fields

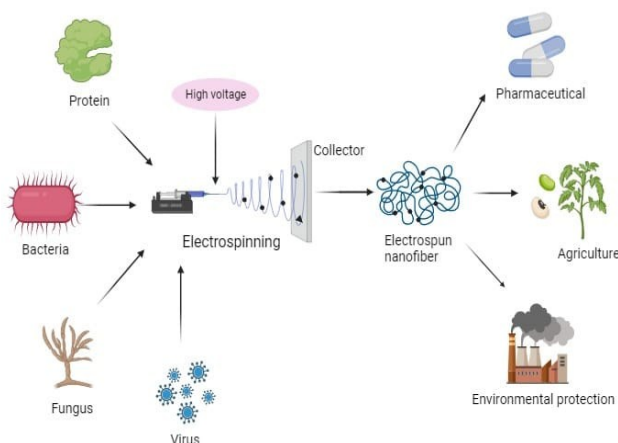


Fig. 3. A schematic process of horizontal electrospinning set up and the application of electrospun nanofibers.

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 (51). Electrospinning techniques are categorized into six types, as shown in Fig. 4 (52) and is illustrated in Table 2(53).

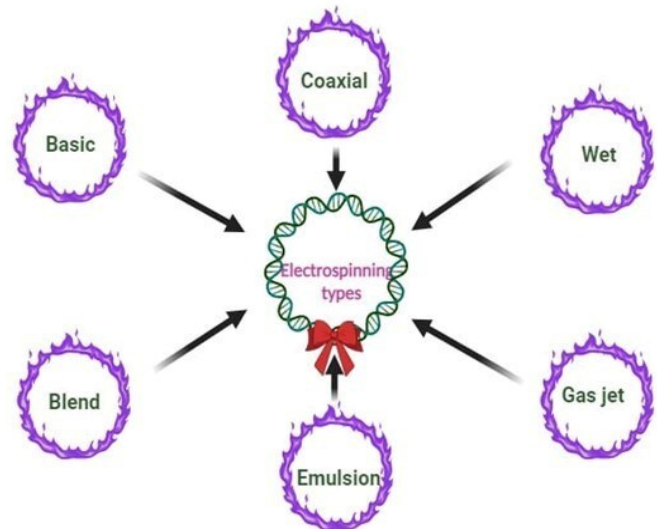


Fig. 4. The given picture depicts the electrospinning types.

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, Kim (58) proposed the development of a new probiotic delivery system. 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).

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

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	53
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.	53
Coaxial Electrospinning	Begins drug release with an initial burst from the sheath, followed by sustained release from the core	54
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	55
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	56
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	57
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.	

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 *Pantoea agglomerans* was shown to improve germination rates, leaf number, leaf length, root dry weight and shoot length in agricultural applications, as demonstrated by De Gregorio et al. (59) in their experimental study.

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. In the study by Gordegir et al. (60), the authors reported using *Gluconobacter oxydans* as a biosensor for glucose detection. 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. de Moraes et al. (61) proposed that *Spirulina* biomass incorporated into polyethylene oxide nanofibers could function as an extracellular matrix for stem cell culture and spinal cord injury treatment. *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 (62).

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 wastewater, 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 wastewater purification, despite being pathogenic to humans. In a study, the fungus was successfully incorporated into nanofibers with a polyvinyl pyrrolidone core and a polyvinylidene fluoride-hexafluoropropylene shell through coaxial electrospinning (26). Once encapsulated, *C. tropicalis* effectively degraded phenols, fermented ethanol in olive mill wastewater 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 polyvinyl alcohol 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 (63).

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. Badrinath et al. (64) 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.

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

Microorganisms	Nanofiber polymer	Purpose	Reference
Bacteria			
<i>Pantoea. agglomerans</i>	Polyvinyl alcohol	Seed coating	(59)
<i>Gloconobacter. Oxydans</i>	Polycaprolactone	Biosensor for Glucose	(60)
<i>Pseudomonas fluorescens</i>	Sodium alginate + soybean oil	Biocontrol of <i>Fusarium solani</i>	(40)
<i>Azospirillum brasilense</i>	Sodium alginate	Plant growth promotion	(104)
<i>Bacillus thuringiensis</i>	Sodium alginate + Chitosan	Insect pest management (<i>Spodoptera litura</i>)	(107)
<i>Methylobacterium aminovorans</i>	Polyvinyl alcohol	Improving germination and growth of plant	(68)
<i>Rhizobium sp.</i>	Gelatin + Arabic gum	Efficiency in nitrogen fixation and promoting plant growth	(5)
<i>Sinorhizobium meliloti</i>	Canola oil + xanthan gum	Nodulation and plant growth promotion	(101)
<i>Spirulina sp.</i>	Poly hydroxybutyrate	Food packaging	(62)
Fungi			
<i>Trichoderma viride</i>	Polyethylene oxide, chitosan	Inhibit the growth of phytopathogenic strains such as <i>Fusarium</i> and <i>Alternaria</i>	(25)

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. Salalha (65) 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. 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 *Pantoea agglomerans* ISIB55 and *Burkholderia caribensis* ISIB40 in spinning solution and nanofibers was determined using the plate dilution method on yeast mannitol agar (59). The sustainability of both strains was measured in terms of log₁₀ colony-forming units per gram.

According to the work by Fung et al. (66), polymers are excellent carriers for encapsulation bacteria, thereby supporting bacterial sustainability. 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 (67).

Mukiri et al. (68) examined the sustainability of *Methylobacterium aminovorans* cells entrapped in polyvinyl alcohol nanofibers. 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 in the work by Kumuthan et al. (47). 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.

The sustainability of a microbial consortium (*Bacillus subtilis* and *Serratia marcescens*) encapsulated in nanofibers was noted to decline when stored at room temperature (69). Polyvinyl alcohol/chitosan nanofibers protected the microorganisms from external environmental conditions (47). The encapsulation of *Azospirillum brasilense* and *Pseudomonas fluorescens* in a polymer mixture-maintained cell viability at 10⁹ CFU/g and 10⁸ CFU/g, respectively, after 12 months of storage at optimal temperatures (70). Similarly, the sustainability of *Lactobacillus acidophilus* (71), *Bradyrhizobium japonicum* (11) and *Pantoea agglomerans* (59), when entrapped in PVA nanofibers, was maintained for extended periods under ambient temperatures storage, as reported by the authors.

Kumuthan et al. (47) concluded that nanofibers are the best carriers for encapsulating microorganisms, effectively maintaining the sustainability of microbial cultures. The polymers used in nanofiber fabrication protect cellular integrity when exposed to external environmental conditions. The average load of *Bacillus subtilis* cells suspended in the polymeric spinning solution was 10¹⁶ CFUs. After the spinning process, 14.06 log₁₀ 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 polyvinyl alcohol and polyvinyl pyrrolidone nanofibers could extend the viability of the bacterium for up to 85-90 days (71). The sustainability of this culture increased to 78.6-90% when encapsulated in agro-waste-based nanofibers. The viability of *Trichoderma viride* spores was preserved when encapsulated in nanofibers composed of polyethylene oxide, 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 (72).

Applications of electrospinning nanofiber in agriculture

The applications of electrospun nanofibers in agriculture encompass seed coating (73), plant protection through the entanglement of fungicides (74) and the entrapment of pesticides (75), herbicides (76) and agrochemicals such as fertilizers (77) and phytohormones (78). 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.

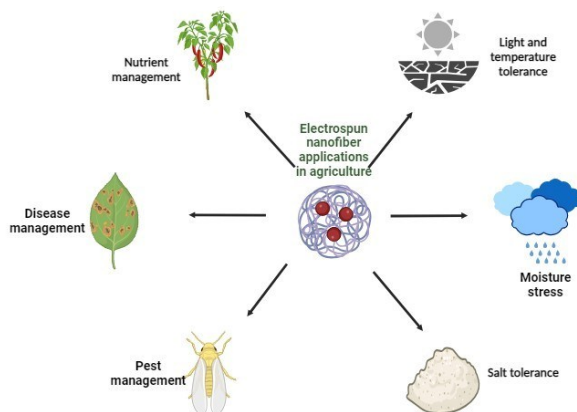


Fig. 5. Electrospun nanofibers application in agriculture.

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 (79). In the work by Farias et al. (31), two active ingredients, abamectin and fluopyram, were encapsulated separately into the nanofibers. 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 nematicide (80, 81).

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 (80, 81).

Preliminary bioavailability experiments by Avenot et al., and Chawla et al. (80, 81) demonstrated the effectiveness of abamectin-loaded nanofibers in immobilizing nematodes using a roundworm (*Caenorhabditis elegans*)-based bioassay. 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 from pathogens and pests. The biocontrol agents and beneficial microbes were entangled in

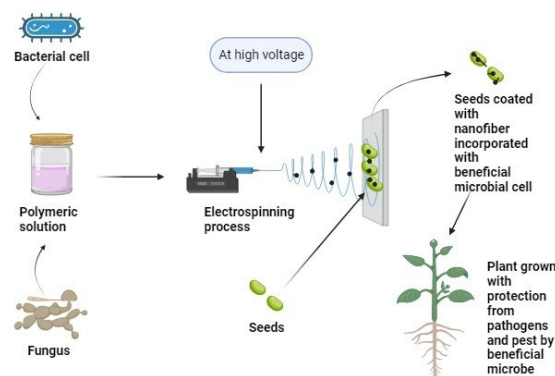


Fig. 6. Nanofibers used in plant protection from pathogens and pests.

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 *Bacillus megaterium* PB50 and *B. pumilus* PB18 exhibit antagonistic activity against fungal pathogens (82). 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 (83).

Moisture stress

Drought is a significant limiting factor in agriculture, obstructing crop growth, reducing productivity and affecting both morphological and physiological processes (84). Water scarcity presents a key challenge for plant growth, constraining crop yields on arable land (85). Prolonged drought periods and a lack of water can render crops unsustainable. Drought conditions lead to yield losses, contributing to severe food shortages (86). 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 (87). 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 ZEBA 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 (88). Coated seeds exhibited improved growth and development under low seeding rates and drought conditions. Similarly, (89) found that a polyethylene glycol (PEG 8000) polymer coating on canola seeds mitigated the effects of moisture stress by enhancing germination in laboratory conditions.

Bacillus 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 (90).

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 (91). 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 (92). Incorporating hydrogels into soil enhances water and fertilizer retention while increasing soil aeration (93).

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 (94). 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 (95). 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 (96). 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 (97). 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 (98). 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 (99). 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

AA and KG carried out the collection of information, drafting, editing, formatting and analysis of the manuscript. AA, KG and GM participated in the sequence alignment and drafted the manuscript. KK, AA and KG assessed the data provided. PM, AA and KG provided the methodology for writing the manuscript. KJ, AA and KG participated in its design and coordination. AP, AA and KG 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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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.

References

- Krishnamoorthy V, Rajiv S. Potential seed coatings fabricated from electrospinning hexaaminocyclotriphosphazene and cobalt nanoparticles incorporated polyvinylpyrrolidone for sustainable agriculture. *ACS Sustainable Chem Eng.* 2017;5(1):146-52. <https://doi.org/10.1021/acssuschemeng.6b01088>
- Lesueur D, Deaker R, Herrmann L, Bräu L, Jansa J. The production and potential of biofertilizers to improve crop yields. In: Arora N, Mehnaz S, Balestrini R. (eds). *Bioformulations: For Sustainable Agriculture*. Springer, New Delhi. 2016;71-92. https://doi.org/10.1007/978-81-322-2779-3_4
- Chaudhary T, Dixit M, Gera R, Shukla AK, Prakash A, Gupta G, Shukla P. Techniques for improving formulations of bioinoculants. *3 Biotech.* 2020;10:199. <https://doi.org/10.1007/s13205-020-02182-9>
- Vassilev N, Vassileva M, Lopez A, Martos V, et al. Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. *Appl Microbiol Biotechnol.* 2015;99:4983-96. <https://doi.org/10.1007/s00253-015-6656-4>
- Rocha I, Ma Y, Souza-Alonso P, Vosátka M, Freitas H, Oliveira RS. Seed coating: a tool for delivering beneficial microbes to agricultural crops. *Front Plant Sci.* 2019;10:1357. <https://doi.org/10.3389/fpls.2019.01357>
- Guan N, Li J, Shin HD, Du G, Chen J, Liu L. Microbial response to environmental stresses: from fundamental mechanisms to practical applications. *Appl Microbiol Biotechnol.* 2017;101:3991-4008. <https://doi.org/10.1007/s00253-017-8264-y>
- John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D. Bio-encapsulation of microbial cells for targeted agricultural delivery. *Crit Rev Biotechnol.* 2011;31(3):211-26. <https://doi.org/10.3109/07388551.2010.513327>
- Vejan P, Abdullah R, Khadiran T, Ismail S, et al. Role of plant growth promoting rhizobacteria in agricultural sustainability-a review. *Molecules.* 2016;21(5):573. <https://doi.org/10.3390/molecules21050573>
- Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil.* 2014;378:1-33. <https://doi.org/10.1007/s11104-013-1956-x>
- Badgar K, Prokisch J, El-Ramady H. Nanofibers for sustainable agriculture: A short communication. *Egypt J Soil Sci.* 2021;61(3):373-80. <https://doi.org/10.21608/ejss.2021.105877.1477>
- Damasceno R, Roggia I, Pereira C, de Sá E. *Rhizobia* survival in seeds coated with polyvinyl alcohol (PVA) electrospun nanofibres. *Can J Microbiol.* 2013;59(11):716-19. <https://doi.org/10.1139/cjm-2013-0508>
- Chanratana M, Han GH, Melvin Joe M, Roy Choudhury A, Sundaram S, Halim MA, Sa T. Evaluation of chitosan and alginate immobilized *Methylobacterium oryzae* CBMB20 on tomato plant growth. *Arch Agron Soil Sci.* 2018;64(11):1489-502. <https://doi.org/10.1080/03650340.2018.1440390>
- Vassilev N, Vassileva M, Martos V, Garcia del Moral LF, et al. Formulation of microbial inoculants by encapsulation in natural polysaccharides: focus on beneficial properties of carrier additives and derivatives. *Front Plant Sci.* 2020;11:270. <https://doi.org/10.3389/fpls.2020.00270>
- Qu B, Luo Y. Chitosan-based hydrogel beads: Preparations, modifications and applications in food and agriculture sectors-A review. *Int J Biol Macromol.* 2020;152:437-48. <https://doi.org/10.1016/j.ijbiomac.2020.02.240>
- Belščak-Cvitanović A, Komes D, Karlović S, Djaković S, et al. Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin, carrageenan, chitosan and psyllium. *Food Chem.* 2015;167:378-86. <https://doi.org/10.1016/j.foodchem.2014.07.011>
- Dobrinčić A, Balbino S, Zorić Z, Pedisić S, et al. Advanced technologies for the extraction of marine brown algal polysaccharides. *Marine Drugs.* 2020;18(3):168. <https://doi.org/10.3390/md18030168>
- Simó G, Fernández-Fernández E, Vila-Crespo J, Ruipérez V, et al. Research progress in coating techniques of alginate gel polymer for cell encapsulation. *Carbohydr Polym.* 2017;170:1-4. <https://doi.org/10.1016/j.carbpol.2017.04.013>
- Bhattarai DP, Aguilar LE, Park CH, Kim CS. A review on properties of natural and synthetic based electrospun fibrous materials for bone tissue engineering. *Membranes.* 2018;8(3):62. <https://doi.org/10.3390/membranes8030062>
- Mojaveri SJ, Hosseini SF, Gharsallaoui A. Viability improvement of *Bifidobacterium animalis* Bb12 by encapsulation in chitosan/poly (vinyl alcohol) hybrid electrospun fiber mats. *Carbohydr Polym.* 2020;241:116278. <https://doi.org/10.1016/j.carbpol.2020.116278>
- Greiner A, Wendorff JH, Yarin AL, Zussman E. Biohybrid nanosystems with polymer nanofibers and nanotubes. *Appl Microbiol Biotechnol.* 2006;71:387-93. <https://doi.org/10.1007/s00253-006-0356-z>
- Agrahari V, Agrahari V, Meng J, Mitra AK. Electrospun nanofibers in drug delivery: fabrication, advances and biomedical applications. In: Mitra AK, Cholkar K, Mondal A. (Eds). *Emerging Nanotechnologies for Diagnostics, Drug Delivery and Medical*

- Devices. Elsevier. 2017;189-215. <https://doi.org/10.1016/B978-0-323-42978-8.00009-7>
22. Hu X, Liu S, Zhou G, Huang Y, et al. Electrospinning of polymeric nanofibers for drug delivery applications. *J Control Release*. 2014;185:12-21. <https://doi.org/10.1016/j.jconrel.2014.04.018>
 23. Fatih Canbolat M, Tang C, Bernacki SH, Pourdeyhimi B, Khan S. Mammalian cell viability in electrospun composite nanofiber structures. *Macromolecular Bioscience*. 2011;11(10):1346-56. <https://doi.org/10.1002/mabi.201100108>
 24. Lee S, Jin G, Jang JH. Electrospun nanofibers as versatile interfaces for efficient gene delivery. *J Biol Eng*. 2014;8:30. <https://doi.org/10.1186/1754-1611-8-30>
 25. Spasova M, Manolova N, Naydenov M, Kuzmanova J, Rashkov I. Electrospun biohybrid materials for plant biocontrol containing chitosan and *Trichoderma viride* spores. *J Bioact. Compat Polym*. 2011;26(1):48-55. <https://doi.org/10.1177/0883911510391446>
 26. Letnik I, Avrahami R, Rokem JS, Greiner A, et al. Living composites of electrospun yeast cells for bioremediation and ethanol production. *Biomacromolecules*. 2015;16(10):3322-28. <https://doi.org/10.1021/acs.biomac.5b00970>
 27. Zussman E. Encapsulation of cells within electrospun fibers. *Polymers for Advanced Technologies*. 2011;22(3):366-71. <https://doi.org/10.1002/pat.1812>
 28. Persano L, Camposeo A, Tekmen C, Pisignano D. Industrial upscaling of electrospinning and applications of polymer nanofibers: a review. *Macromolecular Materials and Engineering*. 2013;298(5):504-20. <https://doi.org/10.1002/mame.201200290>
 29. Thyavihalli Girijappa YG, Mavinkere Rangappa S, Parameswaranpillai J, Siengchin S. Natural fibers as sustainable and renewable resource for development of eco-friendly composites: a comprehensive review. *Front Mater*. 2019;6:226. <https://doi.org/10.3389/fmats.2019.00226>
 30. Krishnamoorthy V, Elumalai G, Rajiv S. Environment friendly synthesis of polyvinylpyrrolidone nanofibers and their potential use as seed coats. *New J Chem*. 2016;40:3268-76. <https://doi.org/10.1039/C5NJ03008K>
 31. Farias BV, Pirzada T, Mathew R, Sit TL, Opperman C, Khan SA. Electrospun polymer nanofibers as seed coatings for crop protection. *ACS Sustainable Chem Eng*. 2019;7(24):19848-56. <https://doi.org/10.1021/acssuschemeng.9b05200>
 32. Espinoza Márquez E, Soto Zarazúa GM, Pérez Bueno JD. Prospects for the use of electrooxidation and electrocoagulation techniques for membrane filtration of irrigation water. *Environ Process*. 2020;7:391-420. <https://doi.org/10.1007/s40710-020-00439-2>
 33. Ma J, Yu Z, Liu S, Chen Y, Lv Y, Liu Y, et al. Efficient extraction of trace organochlorine pesticides from environmental samples by a polyacrylonitrile electrospun nanofiber membrane modified with covalent organic framework. *J Hazard Mater*. 2022;424(B):127455. <https://doi.org/10.1016/j.jhazmat.2021.127455>
 34. Lim CT. Nanofiber technology: current status and emerging developments. *Prog Polym Sci*. 2017;70:1-7. <https://doi.org/10.1016/j.progpolymsci.2017.03.002>
 35. Vinceković M, Jurić S, Đermić E, Topolovec-Pintarić S. Kinetics and mechanisms of chemical and biological agents release from biopolymeric microcapsules. *J Agric Food Chem*. 2017;65(44):9608-17. <https://doi.org/10.1021/acs.jafc.7b04075>
 36. Vemmer M, Patel AV. Review of encapsulation methods suitable for microbial biological control agents. *Biol Control*. 2013;67(3):380-89. <https://doi.org/10.1016/j.biocontrol.2013.09.003>
 37. Vejan P, Khadiran T, Abdullah R, Ismail S, et al. Encapsulation of plant growth promoting Rhizobacteria-prospects and potential in agricultural sector: a review. *J Plant Nutr*. 2019;42(19):2600-23. <https://doi.org/10.1080/01904167.2019.1659330>
 38. Schoebitz M, López Belchí MD. Encapsulation techniques for plant growth-promoting rhizobacteria. In: Arora N, Mehnaz S, Balestrini R. (eds). *Bioformulations: For Sustainable Agriculture*. Springer, New Delhi. 2016;251-65. https://doi.org/10.1007/978-81-322-2779-3_14
 39. Saberi Riseh R, Skorik YA, Thakur VK, Moradi Pour M, Tamanadar E, Noghabi SS. Encapsulation of plant biocontrol bacteria with alginate as a main polymer material. *Int J Mol Sci*. 2021;22(20):11165. <https://doi.org/10.3390/ijms220211165>
 40. Pour MM, Saberi-Riseh R, Mohammadinejad R, Hosseini A. Investigating the formulation of alginate-gelatin encapsulated *Pseudomonas fluorescens* (VUPF5 and T17-4 strains) for controlling *Fusarium solani* on potato. *Int J Biol Macromol*. 2019;133:603-13. <https://doi.org/10.1016/j.ijbiomac.2019.04.071>
 41. Liffourrena AS, Lucchesi GI. Alginate-perlite encapsulated *Pseudomonas putida* A (ATCC 12633) cells: Preparation, characterization and potential use as plant inoculants. *J Biotechnol*. 2018;278:28-33. <https://doi.org/10.1016/j.jbiotec.2018.04.019>
 42. Chaudhary SA, Patel DM, Patel JK, Patel DH. Solvent emulsification evaporation and solvent emulsification diffusion techniques for nanoparticles. In: Patel JK, Pathak YV.(Eds). *Emerging Technologies for Nanoparticle Manufacturing*. Cham: Springer International Publishing; 2021.287-300. https://doi.org/10.1007/978-3-030-50703-9_12
 43. Zuidam NJ, Shimoni E. Overview of microencapsulates for use in food products or processes and methods to make them. In: Zuidam N, Nedovic V. (eds). *Encapsulation Technologies for Active Food Ingredients and Food Processing*. Springer, New York, NY.2010;3-29. https://doi.org/10.1007/978-1-4419-1008-0_2
 44. Zhang R, Hoffmann T, Tsotsas E. Novel technique for coating of fine particles using fluidized bed and aerosol atomizer. *Processes*. 2020;8(12):1525. <https://doi.org/10.3390/pr8121525>
 45. Tarrés Q, Oliver-Ortega H, Boufi S, Pèlach MÀ, et al. Evaluation of the fibrillation method on lignocellulosic nanofibers production from eucalyptus sawdust: A comparative study between high-pressure homogenization and grinding. *Int J Biol Macromol*. 2020;145:1199-207. <https://doi.org/10.1016/j.ijbiomac.2019.10.046>
 46. Li Y, Yu J, Ding B. Facile and ultrasensitive sensors based on electrospinning-netting nanofibers/nets. In:Macagnano A, Zampetti E, Kny E. (eds). *Electrospinning for High Performance Sensors*. Springer, Cham. 2015;1-34. https://doi.org/10.1007/978-3-319-14406-1_1
 47. Kumuthan MS, Lakshmanan A, Sabarinathan KG, Subramanian KS, et al. Immobilization and characterization of *Bacillus subtilis* in PVA-chitosan composite Nanofiber. *Pharma Innovation*. 2021;10(12):1541-45. <https://doi.org/10.22271/tpi.2021.v10.i12v.9616>
 48. Wen P, Zong MH, Linhardt RJ, Feng K, Wu H. Electrospinning: A novel nano-encapsulation approach for bioactive compounds. *Trends Food Sci Technol*. 2017;70:56-68. <https://doi.org/10.1016/j.tifs.2017.10.009>
 49. Xue J, Wu T, Dai Y, Xia Y. Electrospinning and electrospun nanofibers: Methods, materials and applications. *Chem Rev*. 2019;119(8):5298-415. <https://doi.org/10.1021/acs.chemrev.8b00593>
 50. Noruzi M. Electrospun nanofibres in agriculture and the food industry: a review. *J Sci Food Agric*. 2016;96(14):4663-78. <https://doi.org/10.1002/jsfa.7737>
 51. Agarwal S, Wendorff JH, Greiner A. Use of electrospinning technique for biomedical applications. *Polymer*. 2008;49(26):5603-21. <https://doi.org/10.1016/j.polymer.2008.09.014>
 52. Shahriar SS, Mondal J, Hasan MN, Revuri V, et al. Electrospinning nanofibers for therapeutics delivery. *Nanomaterials*. 2019;9(4):532. <https://doi.org/10.3390/nano9040532>
 53. Tipduangta P, Belton P, Fabian L, Wang LY, Tang H, Eddleston M,

- Qi S. Electrospun polymer blend nanofibers for tunable drug delivery: the role of transformative phase separation on controlling the release rate. *Mol Pharmaceutics*. 2016;13(1):25-39. <https://doi.org/10.1021/acs.molpharmaceut.5b00359>
54. Lu Y, Huang J, Yu G, Cardenas R, et al. Coaxial electrospun fibers: applications in drug delivery and tissue engineering. *Wiley Interdiscip Rev: Nanomed Nanobiotechnology*. 2016;8(5):654-77. <https://doi.org/10.1002/wnan.1391>
 55. Lian H, Meng Z. Melt electrospinning vs. solution electrospinning: A comparative study of drug-loaded poly (ϵ -caprolactone) fibres. *Mater Sci Eng C*. 2017;74:117-23. <https://doi.org/10.1016/j.msec.2017.02.024>
 56. Lin Y, Yao Y, Yang X, Wei N, et al. Preparation of poly (ether sulfone) nanofibers by gas-jet/electrospinning. *J Appl Polym Sci*. 2008;107(2):909-17. <https://doi.org/10.1002/app.26445>
 57. Zhang C, Feng F, Zhang H. Emulsion electrospinning: Fundamentals, food applications and prospects. *Trends Food Sci Technol*. 2018;80:175-86. <https://doi.org/10.1016/j.tifs.2018.08.005>
 58. Kim YC, Kim YH, Kim JW, Ha KY. Transplantation of mesenchymal stem cells for acute spinal cord injury in rats: comparative study between intralesional injection and scaffold-based transplantation. *J Korean Med Sci*. 2016;31(9):1373. <https://doi.org/10.3346/jkms.2016.31.9.1373>
 59. De Gregorio PR, Michavila G, Ricciardi Muller L, de Souza Borges C, et al. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. *Plos One*. 2017;12(5):e0176930. <https://doi.org/10.1371/journal.pone.0176930>
 60. Gordegir M, Oz S, Yezer I, Buhur M, et al. Cells-on-nanofibers: Effect of polyethyleneimine on hydrophobicity of poly- ϵ -caprolactone electrospun nanofibers and immobilization of bacteria. *Enzyme Microb Technol*. 2019;126:24-31. <https://doi.org/10.1016/j.enzmictec.2019.03.002>
 61. de Morais MG, Stillings C, Dersch R, Rudisile M, Pranke P, et al. Preparation of nanofibers containing the microalgae *Spirulina* (Arthrospira). *Bioresour Technol*. 2010;101(8):2872-76. <https://doi.org/10.1016/j.biortech.2009.11.059>
 62. Kuntzler SG, de Almeida ACA, Costa JAV, de Morais MG. Polyhydroxybutyrate and phenolic compounds microalgae electrospun nanofibers: A novel nanomaterial with antibacterial activity. *Int J Biol Macromol*. 2018;113:1008-14. <https://doi.org/10.1016/j.ijbiomac.2018.03.002>
 63. Moustafa M, Taha T, Elnouby M, El-Deeb N, et al. Potential detoxification of aflatoxin B2 using *Kluyveromyces lactis* and *Saccharomyces cerevisiae* integrated nanofibers. *Biocell*. 2017;41(2&3):67. <https://doi.org/10.32604/biocell.2017.41.067>
 64. Badrinath N, Jeong YI, Woo HY, Bang SY, Kim C, Heo J. Local delivery of a cancer-favoring oncolytic vaccinia virus via poly (lactic-co-glycolic acid) nanofiber for theranostic purposes. *Int J Pharm*. 2018;552(1-2):437-42. <https://doi.org/10.1016/j.ijpharm.2018.10.020>
 65. Salalha W, Kuhn J, Dror Y, Zussman E. Encapsulation of bacteria and viruses in electrospun nanofibers. *Nanotechnology*. 2006;17(18):4675. <https://doi.org/10.1088/0957-4484/17/18/025>
 66. Fung WY, Yuen KH, Liong MT. Agrowaste-based nanofibers as a probiotic encapsulant: Fabrication and characterization. *J Agric Food Chem*. 2011;59(15):8140-47. <https://doi.org/10.1021/jf2009342>
 67. Rasulov BA, Paerhati P, Yarbekov A, Pattaeva MA, et al. Biofabrication of Cu/Cu2O-Nanoparticles and Exopolysaccharide of *Azotobacter chroococcum* XH2018 Based Nanobiofungicide and Its Characterization. *BioNanoScience*. 2024;4:1-10. <https://doi.org/10.1007/s12668-024-01528-4>
 68. Mukiri C, Raja K, Senthilkumar M, Subramanian KS, et al. Immobilization of beneficial microbe *Methylobacterium aminovorans* in electrospun nanofibre as potential seed coatings for improving germination and growth of groundnut *Arachis hypogaea*. *Plant Growth Regul*. 2022;97:419-27. <https://doi.org/10.1007/s10725-021-00737-1>
 69. Hussain Z, Khan MA, Iqbal F, Raffi M, Hafeez FY. Electrospun microbial-encapsulated composite-based plasticized seed coat for rhizosphere stabilization and sustainable production of canola (*Brassica napus* L.). *J Agric Food Chem*. 2019;67(18):5085-95. <https://doi.org/10.1021/acs.jafc.8b06505>
 70. Perez JJ, Francois NJ, Maroniche GA, Borrajo MP, Pereyra MA, Creus CM. A novel, green, low-cost chitosan-starch hydrogel as potential delivery system for plant growth-promoting bacteria. *Carbohydr Polym*. 2018;202:409-17. <https://doi.org/10.1016/j.carbpol.2018.07.084>
 71. Nagy ZK, Wagner I, Suhajda Á, Tobak T, et al. Nanofibrous solid dosage form of living bacteria prepared by electrospinning. *Express Polym Lett*. 2014;8(5):352-61
 72. Bhutto MA, Bhutto MA, Mangrio GS, Charan TR, et al. Study on the viability and sustainable release of rice rhizobacteria (*Paenibacillus* IBGE-MAB1) immobilized in nanofibers for enhanced rice seed coating and germination. *BioNanoSci*. 2024;14:3274-285. <https://doi.org/10.1007/s12668-024-01460-7>
 73. Sivalingam S, Kunhilintakath A, Nagamony P, Paspulathi Parthasarathy V. Fabrication, toxicity and biocompatibility of *Sesamum indicum* infused graphene oxide nanofiber-a novel green composite method. *Appl Nanosci*. 2021;11:679-86. <https://doi.org/10.1007/s13204-020-01596-4>
 74. Osanloo M, Arish J, Sereshti H. Developed methods for the preparation of electrospun nanofibers containing plant-derived oil or essential oil: a systematic review. *Polym Bull*. 2020;77:6085-104. <https://doi.org/10.1007/s00289-019-03042-0>
 75. Meraz-Dávila S, Pérez-García CE, Feregrino-Perez AA. Challenges and advantages of electrospun nanofibers in agriculture: a review. *Mater Res Express*. 2021;8(4):042001. <https://doi.org/10.1088/2053-1591/abee55>
 76. Mehrani Z, Ebrahimzadeh H, Moradi E. Use of aloin-based and rosin-based electrospun nanofibers as natural nanosorbents for the extraction of polycyclic aromatic hydrocarbons and phenoxyacetic acid herbicides by microextraction in packed syringe method prior to GC-FID detection. *Microchim Acta*. 2020;187:401. <https://link.springer.com/10.1007/s00604-020-04374-9>
 77. Noeaid P, Chuysinuan P, Pitakdantham W, Aryuwananon D, et al. Eco-friendly polyvinyl alcohol/poly(lactic acid) core/shell structured fibers as controlled-release fertilizers for sustainable agriculture. *J Polym Environ*. 2021;29:552-64. <https://doi.org/10.1007/s10924-020-01902-9>
 78. Mirheidari F, Hatami M, Ghorbanpour M. Effect of different concentrations of IAA, GA3 and chitosan nano-fiber on physiological characteristics and metabolite contents in roselle (*Hibiscus sabdariffa* L.). *S Afr J Bot*. 2022;145:323-33. <https://doi.org/10.1016/j.sajb.2021.07.021>
 79. Atila D, Keskin D, Tezcaner A. Cellulose acetate based 3-dimensional electrospun scaffolds for skin tissue engineering applications. *Carbohydr. Polym*. 2015;133:251-61. <https://doi.org/10.1016/j.carbpol.2015.06.109>
 80. Avenot HF, Luna M, Michailides TJ. Phenotypic and molecular characterization of resistance to the SDHI fungicide fluopyram in populations of *Alternaria alternata* from pistachio orchards in California. *Crop Prot*. 2019;124:104838. <https://doi.org/10.1016/j.cropro.2019.05.032>
 81. Chawla S, Patel DJ, Patel SH, Kalasariya RL, Shah PG. Behaviour and risk assessment of fluopyram and its metabolite in cucumber (*Cucumis sativus*) fruit and in soil. *Environ Sci Pollut Res*. 2018;25:11626-34. <https://doi.org/10.1007/s11356-018-1439-y>

82. Devarajan AK, Truu M, Gopalasubramaniam SK, Muthukrishnan G, Truu J. Application of data integration for rice bacterial strain selection by combining their osmotic stress response and plant growth-promoting traits. *Front Microbiol.* 2022;13:1058772. <https://doi.org/10.3389/fmicb.2022.1058772>
83. Dutta P, Kumari A, Mahanta M, Upamanya GK, Heisnam P, Borua S, et al. Nanotechnological approaches for management of soil-borne plant pathogens. *Front Plant Sci.* 2023;14:1136233. <https://doi.org/10.3389/fpls.2023.1136233>
84. Hussain S, Hussain S, Qadir T, Khaliq A, Ashraf U, et al. Drought stress in plants: An overview on implications, tolerance mechanisms and agronomic mitigation strategies. *Plant Sci Today.* 2019;6(4):389-402. <https://doi.org/10.14719/pst.2019.6.4.578>
85. Kumar DA, Sabarinathan KG, Kannan R, Balachandar D, et al. Isolation and characterization of drought tolerant bacteria from rice phyllosphere. *Int J Curr Microbiol App Sci.* 2019;8(6):2655-64. <https://doi.org/10.20546/ijcmas.2019.806.319>
86. Maswada HF, Mazrou YS, Elzaawely AA, Eldein SM. Nanomaterials. Effective tools for field and horticultural crops to cope with drought stress: A review. *Span J Agric Res.* 2020;18(2):e08R01. <https://doi.org/10.5424/sjar/2020182-16181>
87. Gorim L, Asch F. Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. *J Agron Crop Sci.* 2012;198(2):81-91. <https://doi.org/10.1111/j.1439-037X.2011.00490.x>
88. Leinauer B, Serena M, Singh D. Seed coating and seeding rate effects on turfgrass germination and establishment. *Horttechnology.* 2010;20(1):179-85. <https://doi.org/10.21273/HORTTECH.20.1.179>
89. Willenborg CJ, Gulden RH, Johnson EN, Shirtliffe SJ. Germination characteristics of polymer-coated canola (*Brassica napus* L.) seeds subjected to moisture stress at different temperatures. *Agron J.* 2004;96(3):786-91. <https://doi.org/10.2134/agronj2004.0786>
90. Arun K D, Sabarinathan KG, Gomathy M, Kannan R, et al. Mitigation of drought stress in rice crop with plant growth-promoting abiotic stress-tolerant rice phyllosphere bacteria. *J Basic Microbiol.* 2020;60(9):768-86. <https://doi.org/10.1002/jobm.202000011>
91. Fu J, Panhuis MiH Hydrogel properties and applications. *J Mater Chem B.* 2019;7:1523-25. <https://doi.org/10.1039/C9TB90023C>
92. Ahmed EM. Hydrogel: Preparation, characterization and applications: A review. *J. Adv. Res.* 2015;6(2):105-21. <https://doi.org/10.1016/j.jare.2013.07.006>
93. Guilherme MR, Aouada FA, Fajardo AR, Martins AF, Paulino AT, Davi MF, et al. Superabsorbent hydrogels based on polysaccharides for application in agriculture as soil conditioner and nutrient carrier: A review. *Eur Polym J.* 2015;72:365-85. <https://doi.org/10.1016/j.eurpolymj.2015.04.017>
94. Hotta M, Kennedy J, Higginbotham C, Morris N, . Durum wheat seed germination response to hydrogel coatings and moisture under drought stress. *Am J Agric Biol Sci.* 2016;11(2):67-75. <https://doi.org/10.3844/ajabssp.2016.67.75>
95. Mangold JM, Sheley RL. Effects of soil texture, watering frequency and a hydrogel on the emergence and survival of coated and uncoated crested wheatgrass seeds. *Ecological Restoration.* 2007;25(1):6-11. <https://doi.org/10.3368/er.25.1.6>
96. Gesch RW, Archer DW. Influence of sowing date on emergence characteristics of maize seed coated with a temperature-activated polymer. *Agron J.* 2005;97(6):1543-50. <https://doi.org/10.2134/agronj2005.0054>
97. Serena M, Leinauer B, Sallenave R, Schiavon M, Maier B. Turfgrass establishment from polymer-coated seed under saline irrigation. *HortScience.* 2012;47(12):1789-94. <https://doi.org/10.21273/HORTSCI.47.12.1789>
98. Ghassemi-Golezani K, Nikpour-Rashidabad N. Seed pretreatment and salt tolerance of dill: osmolyte accumulation, antioxidant enzymes activities and essence production. *Biocatal Agric Biotechnol.* 2017;12:30-35. <https://doi.org/10.1016/j.bcab.2017.08.014>
99. Mahdavi B, Rahimi A. Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *Eurasia J Biosci.* 2013;7:69-76.
100. Barrera MC, Jakobs-Schoenwandt D, Gómez MI, Serrato J, et al. Formulating bacterial endophyte: Pre-conditioning of cells and the encapsulation in amidated pectin beads. *Biotechnol Rep.* 2020;26:e00463. <https://doi.org/10.1016/j.btre.2020.e00463>
101. de Melo BAG, Motta FL, Santana MHA. Humic acids: Structural properties and multiple functionalities for novel technological developments. *Mater Sci Eng C.* 2016;62:967-74. <https://doi.org/10.1016/j.msec.2015.12.001>
102. Bhise KK, Dandge PB. Alleviation of salinity stress in rice plant by encapsulated salt tolerant plant growth promoting bacteria *Pantoea agglomerans* strain KL and its root colonization ability. *Arch Agron Soil Sci.* 2019;65(14):1955-68. <https://doi.org/10.1080/03650340.2019.1584395>
103. Guo L, Wu Z, Rasool A, Li C. Effects of free and encapsulated co-culture bacteria on cotton growth and soil bacterial communities. *Eur J Soil Biol.* 2012;53:16-22. <https://doi.org/10.1016/j.ejsobi.2012.08.003>
104. Zago SL, dos Santos MF, Konrad D, Fiorini A, et al. Shelf life of *Azospirillum brasilense* in alginate beads enriched with trehalose and humic acid. *J Agric Sci.* 2019;11(6):269-80. <https://doi.org/10.5539/jas.v11n6p269>
105. Chi Y, Wang D, Jiang M, Chu S, Wang B, et al. Microencapsulation of *Bacillus megaterium* NCT-2 and its effect on remediation of secondary salinization soil. *J Microencapsul.* 2020;37(2):134-43. <https://doi.org/10.1080/02652048.2019.1705409>
106. John RP, Tyagi RD, Brar SK, Prévost D, Surampalli RY. Effect of emulsion formulation of *Sinorhizobium meliloti* and pre-inoculated seeds on alfalfa nodulation and growth: a pouch study. *J Plant Nutr.* 2013;36(2):231-42. <https://doi.org/10.1080/01904167.2012.739243>
107. Duraimurugan P, Chandrika KS, Bharathi E, Roy DN. Encapsulation of *Bacillus thuringiensis* using sodium alginate and chitosan coacervates for insect-pest management. *Carbohydr Polym Technol Appl.* 2024;8:100540. <https://doi.org/10.1016/j.carpta.2024.100540>