

## **Cellular and Molecular Biology**

#### Review

CMB

# Innovative capsulation and microencapsulation of plant hormones: a strategy to combat plant pathogens

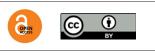


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#### **Article Info**

#### Abstract



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One of the prevailing trends in contemporary agriculture is the application of biological control. Nevertheless, several reports suggest that biocontrol bacteria exhibit poor survival rates in host plants. Consequently, the concept of shielding biological control agents by encapsulating them in outer coatings has gained popularity. Several techniques, including extrusion, spray drying, and emulsification, have been introduced to encapsulate biocontrol bacteria. Much research has focused on the preparation of suitable synthetic hormone products capable of influencing plant growth and development in agriculture. The most effective approach to address this demand is through controlled release systems. One of these techniques involves encapsulating growth hormones. The encapsulation procedure must adhere to crucial standards such as biocompatibility, biodegradability, and provision for sustained viability and performance. Nonetheless, it is essential to conduct further research on the consequences of encapsulation and targeted release in organic farming systems. The creation of a novel composition grounded on biodegradable polymers has the potential to enhance the volume and quality of agricultural yields significantly. The current investigation endeavors to scrutinize the encapsulation of plant hormones and microencapsulation and their effectiveness in counteracting plant pathogens.

Keywords: Encapsulation techniques, Microencapsulation, Pathogen Management, Plant hormones, Plant Pathogens

#### 1. Introduction

Substantial demand for sustenance and other agricultural commodities accompanies the perpetual escalation of the global population. Consequently, the optimization of farming techniques should be further augmented, and the gradual escalation of agrochemicals, especially pesticides, is a necessary measure for crop protection and growth regulation. Nevertheless, the extensive utilization of pesticides has aroused grave apprehensions. There exists a pressing exigency to enhance the efficiency of pesticides to mitigate their deleterious effects on the environment and human health. The enhancement of pesticide efficacy through management is of utmost importance for societal progression and human welfare. Within the category of pesticides, plant growth regulators constitute an essential sector and certain natural plant hormones may function as supplements in the regulation of crop growth and development [1,2]. The employment of controlled release systems is gaining widespread acceptance as an avenue for the administration of agricultural chemicals [3,4]. A significant benefit derived from controlled release systems is their capacity to ensure the appropriate dosage of the active ingredient for the required duration in the surrounding environment. This attribute not only heightens the efficacy of ac-

tive agents but also curtails the introduction of chemicals into the soil, thereby mitigating the potential for environmental harm [5]. One of the fundamental characteristics of controlled release materials is their ability to be easily transported and dispersed on-site utilizing conventional machinery, providing an economic and operational advantage while reducing errors that occur in standard preparation and the use of liquid concentrate. Precision delivery of active components to the most effective point is achievable. Furthermore, controlled-release materials are safer for individuals who handle them or come into contact with treated products during harvest or storage, in contrast to conventional products. There is currently a demand for premixed formulations that combine all primary plant growth factors, including nutrients, plant growth regulators, and protective substances, into a single product [6] Plant cells harbor essential substances that play a pivotal role in the proliferation, maturation, and operational efficacy of the entire organism [7] Plant hormones are not considered to be nutrients, rather they are classified as chemicals which, when present in minute quantities, stimulate the growth, development, and differentiation of cells and tissues [8].

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Phytohormones, which are low molecular weight organic substances of endogenous origin, facilitate the interaction of cells, tissues, and organs by existing in small quantities within tissues. They play an important role in initiating, regulating, and controlling morphogenetic and physiological programs [9]. Phytohormones are present in plants at comparatively low concentrations, prompting numerous research endeavors aimed at developing synthetic hormone products known as plant growth regulators (PGRs) that are capable of influencing plant growth and development in the realm of agricultural practice [10]. The best way to meet this need is through controlled release systems. One of these methods is the encapsulation of growth hormones. Therefore, this research was conducted to investigate the capsule and microencapsulation of plant hormones and their use in combating plant pathogens.

#### 2. Plant hormones

Hormones refer to chemical compounds that function effectively in relatively low concentrations, enabling them to elicit signals and regulate the response and growth of living organisms via the circulation of these compounds in either some or all parts of their bodies [11]. Plant hormones regulate all facets of plant growth and development, including embryogenesis [12], organ size regulation, pathogen defense [13,14], and stress tolerance [15,16] The performance of hormones encompasses procedures for transmitting signals. Signal transduction comprises a process that entails converting intracellular or extracellular signals into cellular responses. These signaling techniques may be categorized into three types based on transmission distances: 1) long-range (plant) or endocrine (animal) signaling, wherein the signal and target cells are considerably distant; 2) paracrine signaling, wherein the signal and target cells are adjacent to each other; and 3) autocrine signaling, wherein the signal and target cells are identical [11]. These hormones are currently classified into five groups, including auxins, cytokinins, ethylene, abscisic acid, and gibberellins. Auxins are produced in stem organs, young leaves, and growing seeds and act by increasing the length of cells and separating leaves, flowers, and older fruits. Gibberellins like auxins are vital for plant growth and are produced in leaves, and young stem tissue, as well as in germinated seeds and fruits. Cytokinin is a plant growth regulator found in tissues where intense cell division occurs and is abundantly found in growing seeds, root tips, leaves, and fruits. The action of abscisic acid, which is mainly produced in the leaves and ends of the root and stem, is different from other hormones because it prevents plant growth. Ethylene is very mobile throughout plant tissues and is essential for ripening fruits and promoting leaf fall [17,18].

#### 3. Encapsulation

Encapsulation constitutes a physical or chemical phenomenon that yields grains of varying sizes, ranging from nanometers to millimeters [19,20] The primary objective of encapsulation lies in enhancing the stability of biological agents while facilitating their efficacy during the use process [21]. Capsule constituents are polymer materials that constitute the capsule matrix. In addition, the capsule frequently comprises one to five additives that exhibit synergistic effects, thereby enhancing product characteristics [22]. Encapsulation of active agents in carrier matrices via microparticles is an advanced technological approach. The utilization of encapsulation offers a host of advantages, including 1) Active agents are safeguarded from the surrounding environment., 2) the diminution of their deterioration whilst employed is mitigated., 3) the amount of active agents are used less, and the most essential fact 4) the feasibility of controlling the delivery of the active agent to plants has been investigated. It has been established that encapsulation in biopolymer matrices is a viable approach for achieving the controlled release of fertilizer. This method has been acknowledged as an efficacious means of achieving controlled release [23]. The selection of an encapsulation system is predominantly influenced by the desired dimensions of the sustained release system, as well as the physical and chemical characteristics of the active ingredient and other associated constituents, including polymers, additives, and inert substances [24,25]. The employment of this methodology is prevalent in the domains of pharmaceutical and food industries for drug administration, flavor enhancement, textile production, storage, packaging, and other similar applications. Recently, the technology in agricultural processes has witnessed improvement through the incorporation of encapsulation techniques, aiming to diminish the employment of hazardous agricultural chemicals, enhance seed germination, and elevate quality. The quality of seeds has been elevated through various measures, such as ameliorated soil quality, regulated nutrient doses, and improved water retention. The encapsulation procedure utilizes biocompatible materials, which may be of either synthetic or natural origin, to enclose chemicals, nutrients, growth stimulants, seeds, and the like. These capsules can be stimulated for release through the utilization of encapsulators, which are attached to a stimulus [26–30]. The encapsulation approach is classified into three categories namely macro, micro, and nanoencapsulation. The dimensions of these particles range from a few micrometers to nanometers and are determined based on the intended application. In some cases, nanoscale size is employed for targeted drug delivery systems in living organisms whereas micro- and macroparticles are suitable for numerous applications [29–31].

#### 4. Encapsulation materials

Numerous investigations have been conducted on diverse materials possessing distinctive properties. The materials employed for encapsulation serve as a covering and are categorized as shell, membrane, matrix, capsule, or carrier depending on their specific usage. The active agent is situated within the core [29,32]. The primary function of the core is to ensure protection of the material from deterioration caused by environmental elements, while simultaneously reducing evaporation and leaching. In addition to this, it is designed to improve its efficacy against harmful microbes, insects, and pests, minimize the environmental risks associated with the material, and promote the movement of hazardous chemicals. The active component can take on a solid, gaseous, or liquid state. Encapsulation has been discovered to be effective in increasing the rate-controlled delivery of the active agent in specific cases and also serves as a protective barrier against external agents [33]. The attributes such as accessibility, degradability, permeability, and the like, are taken into account before the selection of the encapsulating material. The encapsulating agent necessitates the possession

of inhibitory traits to restrict the impact of extraneous elements on the internal molecules [30]. Polymers have been utilized as encapsulants for a considerable duration due to their facile handling, compatibility with nature, non-toxicity, and high permeability. Natural materials employed as encapsulants include chitosan, alginate, lignin, starch, modified polysaccharides, carboxymethyl cellulose, gum arabic, and gelatin, among others, which serve to effectuate targeted and controlled release of macromolecules. However, synthetic polymers such as polyvinyl alcohol, polystyrene, and polyacrylamide have also been employed for analogous purposes. The polymers are tailored to facilitate controlled release based on seasonal conditions, the desired release rate, and the practical application of the formulation, among other factors. These factors aid in minimizing the application of chemicals and their interaction with non-target species, thereby constraining the washing, destruction, and evaporation of the capsule [34,35]. The other classification pertains to superabsorbent polymers that possess elevated storage capacity and are specifically utilized in arid regions. Among the superabsorbents are polyacrylic acid, polyvinyl chloride, and polyacrylamide. Initiating diffusion is a range of stimuli, including pressure, temperature, pH, UV, visible light, enzymes, and microbiota-based chemicals. Hydrogel superabsorbents are produced through the utilization of four distinct methods [36]. Polymerization is a process that involves dissolving monomers in solvents and conducting it in the presence of an initiator at a specific temperature. Application-based encapsulant synthesis also employs emulsion polymerization and photopolymerization. The encapsulator plays a crucial role in maintaining the stability and durability of bioactive molecules, thereby reducing the rate of degradation until the optimal usage time [37].

#### 5. Encapsulation techniques

The attainment of encapsulation for active molecules can be accomplished through the utilization of diverse criteria that are reliant upon the eventual outcome sought. If a particular form and dimension are essential for the ultimate product, suspension polymerization can be employed. Simultaneously, hydrophilic polymers have the potential to be converted into hydrophobic polymers by cross-linking them with other polymers possessing the appropriate properties. Consequently, the selection of materials and synthesis methods is predicated upon the intended purpose [38]. The underlying objective of encapsulation is to achieve immobilization, protection, stability, performance, and controlled release. Its applications have been identified in various industries including food, cosmetics, agriculture, and pharmaceuticals. The fundamental principle of encapsulation comprises three distinct stages. Firstly, the nature of the core needs to be determined, whether it is in a solid or liquid state. Solid cores encompass a matrix that contains either powder or encapsulated crystals, which are coated with solvents, surfactants, suspensions, and emulsions to form liquid cores. Following this, the next stage involves dispersing liquid nuclei through the spray method, which leads to the formation of emulsion or droplet extrusion [30,39]. The process of spray drying entails the introduction of a nebulized slurry into a vertical chamber, through which hot air is circulated. The resultant product is then collected at the bottom of the section. Similarly, spray cooling involves the use of cold air circulation to solidify the product [40,41]. Droplet extrusion represents an additional technique that contributes to the encapsulation process. The substance that is intended to be enclosed is amalgamated with the encapsulator and then conveyed through a mold corresponding to the desired dimensions and appearance. The outcome consists of a solution encased within a rigid shell. Generally, this approach is implemented to encapsulate seeds to prolong their lifespan. Sodium alginate and calcium chloride can be utilized as the encapsulator and hardening solution for this purpose. Moreover, droplet extrusion bears significance in the synthesis of urea-based fertilizers [42]. The coacervation method is involved in encapsulating the herbicide. This method includes polymer deposition around a macromolecule through changes in the physicochemical properties of the solution [43].

#### 6. Encapsulation of plant hormones

The creation of micro and nanoparticles as drug encapsulation agents is regarded as a highly auspicious method to enhance the efficacy of plant hormone utilization. The utilization of nano-microparticle carriers for the encapsulation of plant hormones is generally associated with numerous benefits: 1) enhancing the stability of plant hormones through safeguarding them against photolytic, chemical, and microbial degradation. 2) reducing the use of plant hormones through controlled release 3) Reducing environmental hazards and 4) increasing operational efficiency [44-46]. Various encapsulation materials, comprising both inorganic materials, including silica, metal, and metal oxides, as well as organic materials, such as chitosan and starch, have been employed in the synthesis of plant hormones [47–53]. In comparison to the unbound state, hormones that are encapsulated exhibit greater effectiveness in regulating the growth and development of plants [54] However, a substantial drawback of the encapsulation technique pertains to the lack of environmental sustainability exhibited by the majority of the encapsulation materials employed, such as graphene and polyethylene glycol. Furthermore, the process of preparing nanoparticles is characterized by a certain degree of complexity and financial burden [55]. Thus, it is crucial to establish uncomplicated encapsulation techniques employing economical and ecologically sustainable substances for the production of multifunctional plant hormone formulations, including anti-phytotoxicity and regulated release. Biopolymers derived from natural origins exhibit immense potential as encapsulating agents in safeguarding plant hormones and extending their activity. In this domain, cellulose, starch, chitosan, alginate, and lignin have been utilized owing to their qualities of biodegradability, ease of accessibility, cost-effectiveness, and non-hazardous nature [52,53,56,57]. Ghorbani et al (2024) showed the encapsulation of indole-3-butyric acid (IBA) hormone in alginate chitosan nanocapsules made it stable and increased the root growth of tobacco plants [4]. Quiñones et al (2010) investigated the modified release profiles of two synthetic analogs of brassinosteroids (DI-31 and S-7) encapsulated in chitosan microspheres they compared them with the release of several dicarboxylic acid steroids (diosgenin mono succinate (DMS), compared diaconate (monogeninite). DMI and diosgenin mono maleate (DMM) were encapsulated in chitosan microspheres. Release profiles in water and methanol were determined. Modified release

of steroids, with a nearly constant release rate during the first ten h, was observed Since steroids are more soluble in alcohol, more incredible release was observed using methanol instead of water. Despite the similarity between the steroids in terms of chemical structure, there were substantial differences between the release profiles due to different degrees of interaction with the chitosan matrix [58]. Shuangbin and colleagues (2022) conducted an investigation into the hormonal profiling of Cymbidium Chinensis' encapsulated and non-encapsulated rhizomes in different storage environments across three distinct settings: 25 °C light, 25 °C dark, and 4 °C dark. A total of 55 endogenous plant hormones were identified, and categorized into six classes namely ABAs, AXs, CKs, GAs, Jas, and SAs. Through the application of PCA and HCA, it was demonstrated that the hormonal profiles of rhizomes in various environments could be classified into three control groups, the encapsulation group, and the non-encapsulation group. In comparison to the control group, AXs (IAA-Asp and IAA-Glc), CKs (2MeScZR and K9G), and GAs (GA19 and GA53) were significantly reduced, while ABAs (ABA-GE) and CKs (cZ9G, DZ, and mT) were dramatically increased across all experimental groups. Furthermore, ABAs (ABA) and CKs (BAP) were specifically reduced in the encapsulation group, whereas CKs (BAPR) were specifically reduced and AXs (IAA-Ala) and CKs (tZOG) were increased in the non-encapsulated group. A comparison of the encapsulation group and the non-encapsulation group revealed that AXs (ICALd) and CKs (BAP and pT) were specifically increased in the nonencapsulation group, while CKs (BAPR) and JAs (OPC-4 and JA) were specifically increased in the encapsulation group [59]. The capsule wall has been engineered to prevent the discharge of nuclear materials into the external environment until the appropriate time. This particular approach serves to safeguard delicate food components such as flavors, vitamins, or salt from the detrimental effects of water, oxygen, or light. Additionally, it is a challenging substance to manipulate in its liquid state, as it transforms into a powder that dissolves with ease in the water. Encapsulation of microorganisms is characterized as a technique for encapsulating solids, liquids, or gaseous materials on a smaller scale. The contents of the capsules are discharged based on specific conditions [60].

#### 7. Encapsulation in combating plant pathogens

Biological control has emerged as a favored trend in modern agriculture. Nevertheless, numerous reports have indicated that the survival rate of biocontrol bacteria in host plants is unsatisfactory. Consequently, the notion of encapsulating biological control agents within external coatings to afford them protection has gained popularity [61]. Chemical control is not only not economical, but often does not show the necessary effectiveness against soil pathogens [62]. Furthermore, the persistent application of chemical control techniques promotes the evolution of pathogenic strains that are immune to these methods, thereby compromising the standard of food production and environmental conditions. Over the past twenty years, the utilization of plant probiotic microorganisms has surfaced as a highly auspicious novel approach to managing plant pathogens [63] as part of establishing safe, low-cost, and low-risk methods for integrated management [64]. Encapsulation of bacteria represents a pragmatic technology that confers a protective barrier around plant probiotic bacterial cells and thereby guarantees their endurance over extended periods through gradual release into the soil. In effect, encapsulated biocontrol bacteria are more efficacious than their liberated counterparts in the management of plant pathogens, principally owing to their sustained viability in challenging environmental circumstances [65]. Bioencapsulation represents an efficacious formulation that protects soil microorganisms while concurrently controlling their gradual and consistent release [66]. The enhancement of these formulations can be achieved through two distinct approaches, namely the provision of nutrients for microbial growth and the utilization of bio-composite capsules that can effectively augment the number of encapsulated bacteria that are being inoculated. The process of encapsulation of microbial inoculations has been observed to have several distinct advantages, including but not limited to safeguarding the microorganisms against mechanical stress and adverse environmental conditions within the soil, facilitating the controlled release of the microorganisms, as well as reducing the likelihood of contamination during transportation and storage [67]. Biopolymers find extensive application in diverse industries, particularly in agriculture, where they are utilized for encapsulation purposes. One such instance is the utilization of ALG-bentonite encapsulation for Pseudomonas putida Rs198. The encapsulated microorganism exhibits superior survivability and effective colonization abilities [68]. Encapsulation techniques of bacteria serve to establish a tangible partition between the internal and external materials, thereby shielding them from the impact of environmental factors such as fluctuations in humidity, shifts in pH, and oxidation [69] The present methodology exhibits numerous benefits, among which is the safeguarding of the contained substance from alterations in the environment and the regulated dissemination of said substance [70]. Encapsulation of microorganisms can protect them from biotic and abiotic stresses [71] this is among the latest and most effective methodologies. By utilizing encapsulated cells in conjunction with a controlled system, the gradual release of microorganisms into the soil is possible, thereby prolonging the efficacy of the approach [72]. Encapsulation has been observed to effectively stabilize and preserve cells in the soil against both biotic and abiotic stresses. Various studies have cited diverse encapsulation techniques as a means of enhancing microbial viability [60,71,73,74] Starch has been a preferred coating material for encapsulating various pesticides, including vegetable oils [75], monoterpenes [76] and 2, 4, 5-trichlorophenoxyacetic acid [77] herbicides are a category of pesticides that are implemented to control and eradicate unwanted plants. Other active molecules, such as neem seed oil and chlorpyrifos, are also utilized for this purpose. The efficacy of these substances has been investigated with alterations in encapsulation techniques. Specifically, cross-linking of starch, guar gum, and both with urea formaldehyde has been studied [28]. Cyclodextrin, a biopolymer generated via enzymatic conversion of starch, is frequently employed in the encapsulation process. It has been determined that including cyclodextrin enhances the physicochemical characteristics of pentazocine a herbicide, promoting superior herbicidal activity [78].

Microencapsulation is a technology for packaging materials in small capsules that can release these materials at controlled rates under specific conditions [79]. Microcapsules consist of a semipermeable, spherical, thin membrane with a strong membrane around a core, which can be solid or liquid [80]. Enclosing microorganisms within polymers represents a specialized area within the field of bacteria carrier technology. Recently, smart polymers have emerged as a novel methodology to encapsulate certain chemical compounds, including antibiotics. Encouragingly, investigational studies have demonstrated their capacity for controlled release within the human body [81]. Nanoencapsulation of pesticides has also been introduced as a smart technology. Pesticides are used to limit the growth of pests [82] Advancements in technology have facilitated the micro-encapsulation of herbicides. Investigations have been carried out regarding the application of urea-formaldehyde microcapsules as a means of encapsulating the widely used pesticide Stokler. In a particular study, the microcapsules were subjected to modifications through the utilization of calcium-based lignosulfonate microcapsules [83] for higher encapsulation efficiency and reduced particle size [26]. Chitosan, a natural biopolymer, has been employed as a coating material for encapsulation purposes. To ensure stability, fertilizers containing nitrogen, potassium, and phosphorus are coated with chitosan. Additionally, a superabsorbent polymer gel is utilized as the outer coating to retain water [84]. The coatings in question confer stability, a decreased release rate, and protection from environmental factors to safeguard the fertilizer from degradation, thereby promoting enhanced interaction with the soil. In a similar way, cellulose and its derivatives have evinced notable efficacy as encapsulating agents [85]. The optimization of the implementation of the aforementioned bioactive agents necessitates the utilization of formulations based on nanotechnology and encapsulation, which augment surface characteristics and yield superior pesticidal effects without jeopardizing human and environmental welfare. To illustrate, the nanoencapsulation of azadirachtin was subjected to emulsification using ricinoleic acid and subsequently coated with carboxymethyl chitosan to enhance the durability and effectiveness of the botanical insecticide in outdoor settings [86]. Various microorganisms, namely diazotrophic bacteria, mycorrhizal fungi, actinomycetes, Pseudomonas, and Trichoderma, have been extensively employed to enhance soil quality and safeguard crops against pests. These microorganisms are enveloped within a suitable agent to protect microbial species and environmental factors that may hinder their functionality. While conventional powder and liquid inoculations have been traditionally used for crop application, microencapsulation has emerged as a novel technology. Amongst the various forms of microencapsulation, microbeads are particularly effective in augmenting cell durability and efficacy by fortifying them against external stressors [87].

#### 8. Discussion and conclusion

The degradation of agricultural chemicals and subsequent decrease in their efficacy due to biotic and abiotic stress can impact yield. To address this, conventional agrochemical application methods have been enhanced through the implementation of an encapsulation process aimed at preserving agrochemicals and preventing overuse and degradation. The agricultural industry has experienced a transformation as a result of technological advancements that have given rise to micro- and nano-tools capable of retaining active molecules and facilitating systemic release. The controlled release can be achieved through external stimuli such as changes in pH and temperature, ion exchange, osmotic and hydraulic pressure, and increased nutrient absorption. Encapsulating biologically and chemically active molecules can lead to better germicidal activity. However, it should be noted that nano encapsulators may also serve as degradation enhancers, and their environmental impact remains unclear [88] Further investigations are imperative to examine the life cycle, exposure to the food chain, decay, and comprehensive influence on living organisms.

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