AgriEngineering

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Review

Trichoderma Production and Encapsulation Methods for Agricultural Applications

Erick Vindas-Reyes [1,*], Randall Chacón-Cerdas [1] and William Rivera-Méndez [2]

[1] Biotechnology Research Center, Applied Biochemistry Laboratory, Biology School, Costa Rica Institute of Technology, Cartago P.O. Box 159-7050, Costa Rica; rchacon@itcr.ac.cr
[2] Biotechnology Research Center, Biocontrol Laboratory, Biology School, Costa Rica Institute of Technology, Cartago P.O. Box 159-7050, Costa Rica; wirivera@itcr.ac.cr
* Correspondence: evindas@itcr.ac.cr

Abstract: *Trichoderma* is one of the most widely used microorganisms in the biological control of plant pathogens. The techniques for its formulation are well known and are commercially distributed in both solid and liquid presentations based on formulations of its reproductive structures. Currently, agricultural systems integrate this type of fungus as an alternative for sustainable production, and even though its traditional formulation still has important limitations, it has a high potential to be combined with new technologies for the development and innovation of products that improve their effectiveness. In response to this, micro- and nanotechnology are presented as alternatives to technify bioagents, promoting greater resistance, viability, and dissemination for both biomass and metabolites through encapsulation and smart delivery techniques. Some works have been developed to achieve this, especially using ionic gelation, with good results for agriculture. In this work, some generalities of the organism are mentioned, including its most common formulations for agricultural applications, information related to encapsulation systems, and the potential for improvement of biologics represented by biomass microencapsulation.

Keywords: biological control; micro- and nanotechnology; bioagent; encapsulation

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Citation: Vindas-Reyes, E.; Chacón-Cerdas, R.; Rivera-Méndez, W. *Trichoderma* Production and Encapsulation Methods for Agricultural Applications. *AgriEngineering* **2024**, *6*, 2366–2384. https://doi.org/10.3390/agriengineering6030138

Academic Editor: Eugenio Cavallo

Received: 4 May 2024
Revised: 20 June 2024
Accepted: 9 July 2024
Published: 22 July 2024

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1. Introduction

Controlling plant pathogens is crucial for maintaining the quality and quantity of food available worldwide, as they affect production systems at various points and with different severity levels [1]. It is estimated that losses in food due to the action of fungi, bacteria, viruses, nematodes, insects, and weeds range from 31% to 42% of the total, which could be higher, possibly doubling this percentage, if the available strategies for controlling plant diseases were disregarded [2]. Among these, fungal problems stand out, with more than 10,000 pathogenic species reported in agriculturally important plants [3].

Various strategies have been tested to achieve this goal, but the inclination for the strong use of chemical products continues to prevail due to cost, availability, and speed of action, regardless of the environmental impact they entail [4,5]. However, the accumulation of toxic substances due to the extensive use of these biocides compromises human health and ecosystems, making them a controversial issue [6,7].

In response to this scenario, the use of biological control agents (BCAs) has positioned itself as one of the main candidates for the development of sustainable agriculture and achieving a change in conventional production methods [8]. These BCAs are usually isolated from the rhizosphere, phyllosphere, or soil near the area where they are intended to be used, thus not adversely altering the local microbiota [9].

BCAs can be made from various organisms, most commonly bacteria and fungi, and selected according to the specific application, the climate where they are going to be implemented, and the growth and storage needs of the biological agent to develop a formulation. Plant-pathogenic bacteria extracts, plant-growth-promoting rhizobacteria,

growth-promoting fungi, non-pathogenic fungi, mycorrhizae, entomopathogenic fungi, mycoparasitic fungi, or endophytic fungi are some of the common groups of microorganisms [8,10,11]. Table 1 shows examples of BCAs and their reported effectivity.

Table 1. Examples of reported fungal and bacterial BCAs since 2020.

BCA Group	BCA Species	Pathogen Group	Pathogen Species	Disease	Crop(s)	Reference
Aerobic Gram-negative antagonistic bacterium	*Pseudomonas protegens*	Aerobic Gram-negative endophytic bacterium	*Pantoea ananatis*	Maize white spot	*Zea mays*	[12]
Aerobic facultative Gram-negative bacterium	*Lysobacter antibioticus*	Obligate aerobic Gram-negative bacterium	*Xanthomonas oryzae* pv. *oryzicola*	Bacterial leaf streak	*Oryza sativa* L.	[11]
Aerobic Gram-positive antagonistic bacterium	*Bacillus amyloliquefaciens*	Aerobic Gram-negative endophytic bacterium	*Ralstonia solanacearum*	Tobacco bacterial wilt	*Nicotiana tabacum* Linne	[13]
Aerobic Gram-positive antagonistic bacterium	*Bacillus subtilis*	Epiphytic fungus Insect	*Stagonospora nodorum* Berk. *Schizaphis graminum* Rond. (pest)	Glume blotch	*T. aestivum*	[14]
Aerobic Gram-positive antagonistic bacterium	*Bacillus thuringiensis*	Endophytic mycoparasitic fungus Insect	*Sclerotinia sclerotiorum* *Plutella xylostella* (pest)	Sclerotiniose	*Brassica campestris* L.	[15]
Aerobic Gram-positive antagonistic bacterium	*Bacillus thuringiensis*	Nematode	*Aphelenchoides besseyi*	Rice white tip	*Oryza sativa* L.	[16]
Aerobic Gram-positive antagonistic bacterium	*Streptomyces antibioticus*	Endophytic fungus	*Neoscytalidium dimidiatum*	Stem canker	*Delonix regia*	[17]
Aerobic chemoorganotropic Gram-negative symbiotic bacterium Aerobic Gram-positive antagonistic bacterium	*Rhizobium* sp. *Bacillus subtilis*	Aerobic Gram-negative endophytic bacterium Epiphytic fungus	*Pseudomonas syringae* *Alternaria solani*	Leaf diseases	*Solanum lycopersicum* L.	[18]
Endophytic fungus	*Aureobasidium pullulans*	Endophytic oomycete Endophytic mycoparasitic antagonistic fungus	*Phytophthora cactorum* *Botrytis cinerea Pers*	Crown rot Root rot Grey mold	*Fragaria* × *ananassa duch.*	[19]
Endophytic fungus	*Induratia* spp.	Endophytic fungus Endophytic mycoparasitic fungus Endophytic fungus	*Colletotrichum lindemuthianum* *Sclerotinia sclerotiorum* *Pseudocercospora griseola*	Anthracnose White mold Angular leaf spot	*Phaseolus vulgaris* L.	[20]
Entomopathogenic fungus	*Beauveria bassiana*	Endophytic fungus Endophytic mycoparasitic antagonistic fungus Insect	*Alternaria alternata* *Botrytis cinerea* *Macrosiphum euphorbiae* (pest)	Spots/Rot on fruits	*Solanum lycopersicum* L.	[21]
Entomopathogenic fungus	*Lecanicillium araneicola*	Insect	*Aphis craccivora* (pest)	Viral vector of barley yellow dwarf virus, papaya ring spot virus, and watermelon mosaic virus	Fruits and cereals	[22]
Mycoparasitic antagonistic/nematicide fungus	*Trichoderma* spp.	Aerobic Gram-negative endophytic bacterium Obligate aerobic Gram-negative bacterium Nematode	*Ralstonia solanacearum* *Xanthomonas campestris* *Meloidogyne incognita* (pest)	Bacterial leaf spot Bacterial wilt Root-Knot nematode	*Solanum lycopersicum* L.	[23]
Mycoparasitic antagonistic fungus	*Trichoderma asperellum*	Endophytic fungus	*Fusarium oxysporum*	Fusarium wilt	*Stevia rebaudiana*	[24]
Mycoparasitic antagonistic fungus Mycoparasitic antagonistic fungus	*Trichoderma longibrachiatum* *Trichoderma asperelloides*	Endophytic fungus	*Magnaporthiopsis maydis*	Late wilt	*Zea mays* L.	[25]

Table 1. *Cont.*

BCA Group	BCA Species	Pathogen Group	Pathogen Species	Disease	Crop(s)	Reference
Mycoparasitic antagonistic fungus	*Trichoderma* sp.	Mycoparasitic antagonistic fungus	*Botrytis cinerea*	Grey mold	*Prunus mume*	[26]
Mycoparasitic antagonistic fungus	*Trichoderma asperellum*	Epiphytic fungus	*Sclerotium cepivorum*	White rot	*Allium cepa* L.	[27]
Mycoparasitic antagonistic fungus	*Coniothyrium minitans* *Trichoderma* spp.	Endophytic fungus	*Sclerotinia sclerotiorum*	Head rot	*Brassica oleracea var. oleracea*	[28]
Mycoparasitic antagonistic fungi Mycorrizae consortium	*Trichoderma harzianum* *Claroideoglomus claroideum* *Claroideoglomus etunicatum* *Funneliformis geosporum* *Funneliformis mosseae* *Glomus micro-aggregatum* *Rhizophagus intraradices*	Endophytic fungus Endophytic fungus	*Fusarium oxysporum* *Verticillium dahliae*	Tomato wilt	*Solanum lycopersicon esculentum* Mill.	[29]

Among filamentous fungi, most of those with BCA capabilities belong to the Ascomycota phylum, and many of its representatives are numerous species of the *Trichoderma* genus [30]. In general, among the biological models for the development of specialized bioinputs for the control of pathogens or pests of agronomic interest, along with the promotion of growth with promising results, the *Trichoderma* sp. genus stands out for its high versatility to adapt to various ecological environments and agricultural scenarios combined with its compatibility with some products used in agricultural management or other biocontrol agents, making it a good candidate for implementation in biological control programs and production systems [31,32]. This trend is also observed in Table 1, where this organism has been implemented in a wide spectrum of agronomic problems. As an interesting fact, in the case of bacterial BCAs, there is a notable preference for the *Bacillus* genus.

2. *Trichoderma* as a Biocontrol Agent

Species of fungi from the genus *Trichoderma* have been used as biocontrol agents for managing diseases and controlling pathogenic fungi in crops [24]. Historically, their versatile management has made them widely recognized as an antagonistic organism since 1920. Furthermore, they possess mechanisms that promote plant growth, induce the production of metabolites of interest and growth-regulating compounds, stimulate plant defense mechanisms, and induce siderophore production [8].

These microorganisms are ubiquitously distributed, rapidly growing on different substrates, and demonstrate swift soil colonization. They exhibit high competition capacity for resources and adaptability to various environments, thanks to their ability to synthesize antimicrobial enzymes and metabolites, their hyperparasitism, and their voracity in acquiring space, oxygen, and nutrients [6]. Identifying a specific species through morphology is challenging due to the plasticity of structural characteristics; hence, molecular testing is always preferable [6].

The mechanisms of action of *Trichoderma* can be divided into two types of relationships: "*Trichoderma*-Plant" and "*Trichoderma*-Pathogens" [33]; the most cited are mycoparasitism (direct attack of other pathogenic fungus by coiling, penetration, and digestion with lytic enzymes), antibiosis (liberation of low-molecular-weight diffusible compounds that inhibit the growth of bacterial or fungal pathogens), competition (effective competitivity for space and nutrients that leads fungal phytopathogens to starvation), and induced resistance (elicit and promote plant defense responses that trigger systemic resistance and systemic acquired resistance) (Figure 1) [34,35]. Besides these, it can facilitate plant–environment interaction, even from plant to plant using phytohormones, help in the absorption of nutrients, and

promote efficient management of internal resources and an intelligent response to abiotic factors, granting *Trichoderma* a growth regulation mechanism [36].

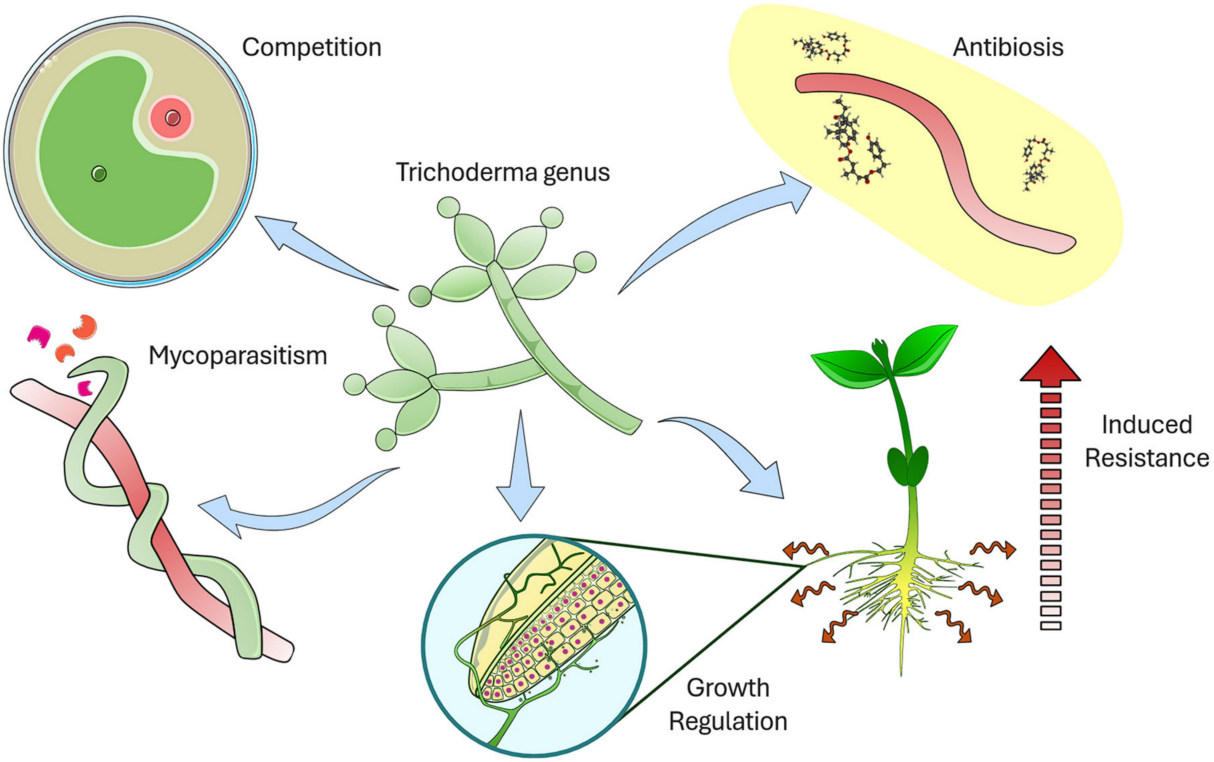


Figure 1. Known mechanisms for *Trichoderma* used in biological control and plant defense.

Examples of these capabilities and their applications are their reported efficacy against pathogens such as *Fusarium oxysporum* [37], *Fusarium graminearum* [38], *Sclerotium rolfsii* [39], *Corynespora cassiicola* [40], *Ralstonia solanacearum* [41], and *Erysiphe alphitoides* [42], among others. This fungus aids the root development of *Phaseolus vulgaris* and *Solanum lycopersicum* plants by facilitating nutrient absorption and mobilization [37,43], improving photosynthetic rate, and reducing oxidation due to metabolism in *Saccharum* sp. crops [44] and promoting systemic defense in *Lactuca sativa* L. [40] and bean plants [43].

In addition, it can produce fungicides or intermediates such as acetamide, dibromocyanacetamide, ethylamide, ethylene glycol, glycine, ethanolamide, citric acid, malic acid, and o-toluic acid. More than 68 secondary metabolites with microbial activity have been identified associated with this fungus [34]. This could be used in the elaboration of alternative products without the need for its original biomass. Finally, there is also an application in the manufacture of pharmaceutical products due to its ability to produce trichosperellins [45].

3. Agricultural Product Formulations of *Trichoderma* sp.

A formulation comprises an active ingredient (AI) (biocontrol agents, natural extracts, chemical molecules) and auxiliary substances or excipients (inert substances that protect and aid the release of the formulation). Excipients ensure the product's stability, protection of the AI from UV radiation, target adhesion, moisture retention, desiccation protection, improved product dispersion, and simplified application [46]. Thus, the primary function of these mixtures is to maintain the microorganism in a state of low or no metabolic activity while keeping it viable and effective for as long as possible during storage [47].

The development and commercialization of BCAs encompass a series of steps, from the idea to the final application. Normally, the microorganism needs prior isolation, analysis,

and optimization of production, usage, and storage conditions before entering the on-field applications phase [48]. Typical challenges associated with those products are:

- Environmental: biotic (interactions with the resident microbiome, alterations of the original effect depending on the crop) and abiotic (variations in soil properties, interactions with other agricultural components).
- Practical: social aspects (added value and perspective from consumers), accessibility (BCA's limited versatility, adaptability, or shelf-life; knowledge of manipulation and application by farmers), and regulations (lack of protocols, guides, laws, or regulations)

Products derived from *Trichoderma* are often able to integrate into a productive chain, thanks to the characteristics of the fungi mentioned before, which alleviates a big part of these challenges. Usually, the formulations contain structures of the living organism, with the aim of establishing it at the site of interest and allowing the fungus the opportunity to express the maximum amount of the mechanisms (Figure 1) on crops and maintain them over time during the fungus's development [49]. Also, when working with fungal BCAs, it is important to take care of the pH, low a_w level, and low temperatures to preserve the product [50].

Substrate selection is a key factor in the production of any BCA-based preparation. When focusing on filamentous fungi like *Trichoderma*, culturing on cereals or low-cost fermentable organic material is a priority due to its practicality for solid substrate fermentations [51]. Broken rice, corn, wheat, soy, sorghum, or waste material from the banana industry are some of the substrates commonly employed for this fungus's multiplication [51,52]. These are processed in volumes less than 1 kg per fermentation in bags or autoclavable containers, and water is added to increase the relative humidity (RH) in the container, promoting optimal development of the microorganism. The temperature range suitable for the growth of this fungus is observed to be 22 to 28 °C, with optimal growth at 25 °C [53]. The process is finally subjected to drying and grinding that can be utilized to recover the conidia used as input when applied directly or in specific mixes that provide additional properties [54].

The use of spores or conidia is recurrent, although using the fungus's biomass in general can be effective. Asexual growth allows obtaining high concentrations of viable reproductive structures that facilitate the dispersal of the organism while conferring the necessary resistance for it to establish itself in the environment [49]. Commercially, it is recommended to use concentrations ranging from 10^5 to 10^{10} spores to achieve adequate action of the BCA [54,55]. It is noteworthy that the resistance, and therefore the lifespan of the spores, is greater when the fungi are cultivated on solid media compared to liquid media, as the stress involved for the organism due to excess moisture and turbulence, as well as the lack of support and surface roughness, impedes the adequate structuring of these bodies [56].

Another significant aspect of this fungus is the reported synergistic activity with another BCA like mycorrhizae [29] or *Bacillus* sp. [57], natural compounds like onion extracts [58], and partial compatibility with chemical pesticides such as Cu-based fungicides [59] or mancozeb [54]. These attributes reduce the chemical dosages typically needed on macroscale systems without compromising its effectiveness as a biocontrol agent [32,60].

The following image (Figure 2) shows a summary of the types and presentations of *Trichoderma* formulations. Each one of them is explained below in their respective section.

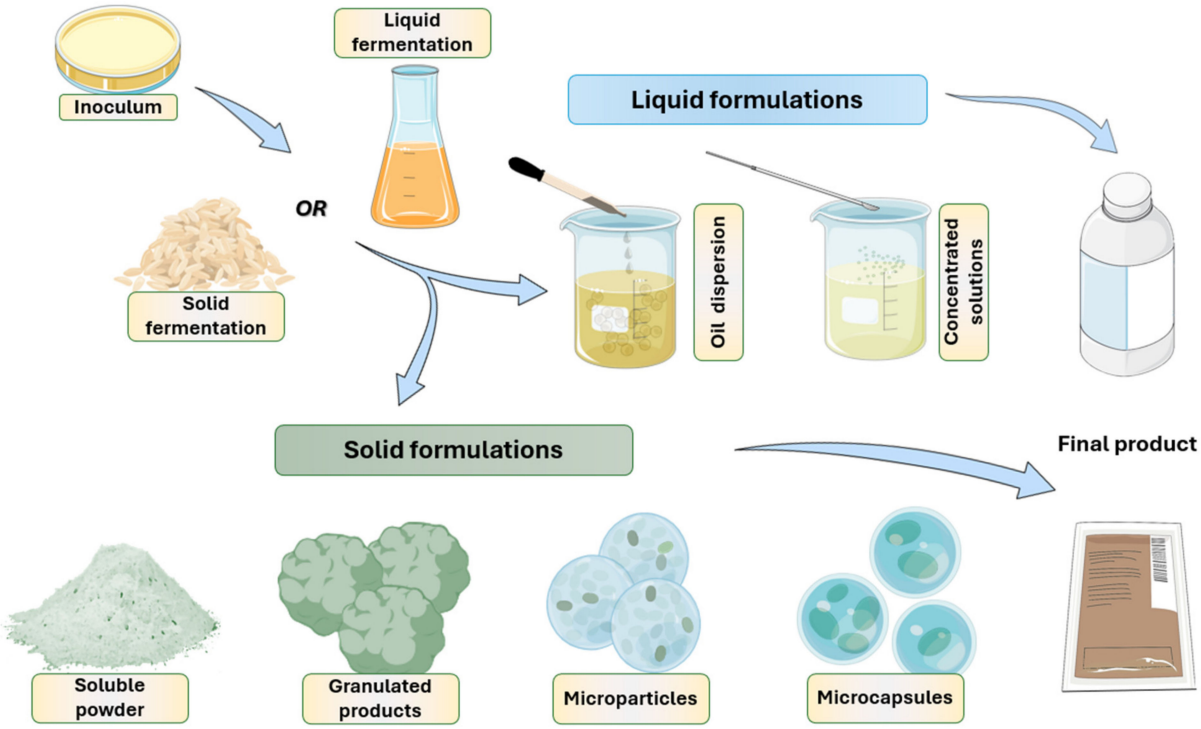


Figure 2. General production techniques of *Trichoderma* formulations.

3.1. Liquid Formulations

Liquid formulations of microbial suspensions, particularly in their dormant state, are enhanced with additives like starch and humic acid to improve viscosity, dispersion, stability, and nutritional properties. These improvements extend shelf life, enhance agricultural suitability, and ensure rapid microorganism contact with plants, boosting their resilience to adverse soil conditions [61]. Compared to conventional carrier-based BCAs, liquid presentations tend to have a longer shelf life (12–24 months) and retain their properties up to 45 °C in extreme cases. They also combat indigenous populations more effectively, are easily identified by their fermented smell, and ensure better survival on seeds and soil, offering ease of application, increased commercial revenue, and higher export potential [62].

The boundaries mentioned are the case for most applications, especially bacterial BCAs, but for fungi such as *Trichoderma*, being exposed for extended periods to a high water content environment decreases their viability due to cell imbibition damage and spontaneous germination [63]. Because of this, there is a need to incorporate specialized carrier substances and protectants that lead to higher-cost production and investigation efforts to develop the product. Additionally, liquid presentations of *Trichoderma* are mostly emulsions with a strong oil base or some type of encapsulated conidia dispersed in water [55]. They also require handling of large work volumes, have limited protection against contamination, and still depend on low temperatures to maintain prolonged stability, representing additional storage costs [64].

3.1.1. Oil Dispersions

An emulsion is defined as an unstable system containing two or more immiscible liquid phases, where one of the phases contains colloidal particles dispersed in the other [65]. For its production, a spore concentrate of the fungus is used, suspended in a combination of immiscible (organic and mineral) solvents in water, and a surfactant is added to eliminate the surface tension of the water. Thus, when combined with water, this allows for the formation of a homogeneous emulsion. A large amount of emulsifier needs to be produced, and the oils used must not be toxic to spores, plant species, animals, or humans. The use

of oils has the advantage of protecting the spores and extending their shelf life, even once applied in the field [51].

3.1.2. Concentrated Suspensions

In aqueous formulations, the biomass of interest is separated and then suspended in a liquid medium that may contain various adjuvants that maintain the stability and physical integrity of the formulation during application. The carrier agent of the active ingredient is water [66]. It must be considered that the product's shelf life may rapidly decrease, as the organism can suffer damage due to the high humidity it is exposed to, and the formula must be complemented with products that help reduce water activity (a_w) [64].

3.2. Solid Formulations

The solid presentation of *Trichoderma*-based products is the dominant commercial alternative. It can be achieved from solid or liquid fermentation, where the biomass is taken with little or no treatment or after a collection and then drying process [51]. In conventional presentations, sometimes part of the initial medium can be integrated into the final formulation without posing a problem; this is determined by the final composition of the product, which is often empirically defined [66].

It is possible to work with various raw materials; costs can be reduced by exchanging inert materials or salts for organic alternatives and waste material, but generally, production costs are high due to the stages involved in their creation, as well as the prior treatment of the active ingredient (AI), but still lower compared to liquid alternatives [54]. There are various methods of application and purposes that each of the different presentations can fulfill. They even allow forming pastes or being applied without alterations as coatings to plant materials, for example, on seeds and seedlings, thus ensuring the accompaniment of the BCA with the crop during its development [67].

Viability tends to be low due to the stress the organism undergoes during processing procedures, packaging, and application. However, while they are easy to store and have a lower risk of contamination compared to liquid presentations, their critical dust content can quickly disperse into the air if not handled properly and could pose a respiratory or other health problem due to conidia and mineral particles [55,66].

3.2.1. Soluble Powders

Hydratable solids containing concentrated conidia or spores are the most common products on the market. They are combined with trace powders such as inert materials, dry plant residues, carbohydrates, oils, and salts that facilitate dispersion and extend the product's shelf life [68]. The materials commonly used to make these powders are talc or clays because they prevent over-hydration of the spores, do not react with the organism's metabolism, and do not complicate their solubilization or form clumps when used in spray applications [68].

3.2.2. Granulated Compounds

Granulated products have a higher degree of environmental and user protection [69]. Thanks to their granular complexion, they are less prone to absorb ambient moisture; hence, they contaminate less thanks to the reduction of water activity and improve the survival of the microorganisms [70]. These products require encapsulation with biocompatible materials that allow the conservation of the spores within their structure. This can be achieved with techniques such as spray-drying or ionic gelation with natural and synthetic polymers. During their elaboration, it is possible to incorporate humectants, dispersants, disintegrants, and adhesives that aid the properties of the granules [69].

3.2.3. Microencapsulation

The microencapsulation of conidia with sugars or lipids increases the survival of fungi such as *Trichoderma*. This process involves creating very small droplets or particles

that contain the BCA and are coated with a protective layer or embedded in a matrix to produce small capsules [71]. Materials used include chitosan, guar gum, gum arabic, sodium alginate, or polyacrylamides. These matrices serve as good transport media and provide different release kinetics, enhancing their efficiency. They physically protect from adverse environmental conditions such as UV radiation or excessive rainfall [72].

They allow for long-term storage at room temperature, reduce the volume needed for production, and facilitate the handling of the product. This is particularly useful for BCAs that require slow releases, making them suitable for soil applications, although this consequently results in a slow rehydration rate, complicating their use when rapid activation of the product is needed [66].

4. Integration with Emerging Technologies

Permanent challenges for sustainable crop production include the optimization and search for efficient agricultural technology that meets production requirements and has a lower environmental impact. Adopting systems focused on biological or biotechnological products is part of this strategy [73].

Micro- and nanotechnology are versatile disciplines that have enabled the optimization of biological products through effective encapsulation of compounds in a broad range of areas, including agricultural biotechnology [74]. This protects the encapsulated active ingredients from the external environment and offers properties such as controlled release of its components or improved interaction with the product's target [75].

These particles can be classified into two types according to their internal structure and morphology: microspheres or microcapsules. A microsphere has its nuclear material or active ingredient randomly distributed throughout the barrier material, while a microcapsule focuses on coating the nuclear material with a polymeric barrier [74]. Each approach offers specific properties that can be exploited according to the application; for example, producing multifunctional products that can reduce microbial load with an antifungal such as copper and almost simultaneously inoculating a beneficial mycoparasitic fungus in the area [76].

Furthermore, these technologies focus on the synthesis of particles from natural polymers through green chemistry. These biopolymers integrate and assimilate rapidly by microorganisms [77]. Another advantage is that both the native polymer and its derivatives provide benefits to the soil and crops as they degrade, functioning analogously to organic matter [78].

Some limitations and technical challenges associated with the encapsulation of BCAs are related to the materials used in their production. Since these bioinputs generally need to exhibit biocompatibility and degradability, the most common choices for encapsulation are natural polymers and derived nanocomposites. However, this presents significant challenges in selecting materials that meet the required mechanical and chemical properties without compromising their effectiveness [79]. For instance, some carbohydrates degrade rapidly due to the action of decomposing microorganisms, which limits their application in processes requiring slow, controlled release while also affecting the shelf life. Another challenge is the combination of materials that not only serve as vehicles for the BCAs but also provide additional functions to the capsule [80].

Standardizing the processes for the characterization and manufacture of encapsulating materials is also a challenge for this technology. Typically, raw material sources are heterogeneous, and extraction/purification protocols are not easily adaptable, negatively impacting the physicochemical characteristics of the resulting encapsulates [80,81]. It is possible that coating materials could impact soil agrobiology, and this remains a research gap, along with toxicity studies that are size-dependent [82].

Regarding regulatory concerns, coordination between technical and governmental sectors is essential for these products to reach the market [83]. The registry of traditional chemical-based products is easier since the formulation is well defined and testable, while the BCA-based ones are commonly a complex matrix with multiple active ingredients [84].

Additionally, it is crucial to streamline processes for the registration and use of these bioinputs in key countries for this kind of technology to grow, like better communication between growers, stakeholders, and decision-makers to cope with the bureaucratic barriers [85]. Europe and the USA have limitations in the registry process due to their focus mainly on additives and microbial content, while China and India are the ones with more formal registered BCAs. China has well-defined categories of BCAs considering multiple features of quality, and India has already included some of the terms (e.g., Biofertilizer) in their legislation and created a proper regulatory mechanism [48]. Concerning commercial production, it is critical to reduce the high costs of research, development, and import expenses, which are already implicit in the BCA market. Scalability is another challenge, primarily due to the high manufacturing costs when encapsulates are produced at the micro- or even nanoscale [86].

4.1. Spore Encapsulation Methods

The method for particle synthesis is largely determined by the defined polymer and the active ingredient's resistance to the physicochemical conditions of the environment in which the reactions will occur. Spores are the preferred material due to their greater resistance compared to other fungal tissues, though other parts, such as the mycelium, can also be employed [49]. Nevertheless, spores have thresholds of temperature, pressure, and acidity they can withstand, which vary according to the organism; hence, tests must be conducted for each case.

Ghosh et al. [87] note that there are numerous technologies for encapsulating nuclear materials, and based on their synthesis methods, they are classified into three groups with their respective techniques:

- Chemical processes: including techniques such as "suspension, dispersion, and emulsion" and "polycondensation".
- Physicochemical processes: "coacervation", "Layer-by-Layer (L-B-L)", "ionic gelation", and "supercritical microencapsulation with CO_2".
- Physical-mechanical processes: "spray-drying", "spraying with multiple nozzles", "fluid bed coating", "centrifugal techniques", "vacuum encapsulation", and "electrostatic encapsulation".

It has been reported that the application of these techniques to enhance the viability of microorganisms is a potential pathway to improve their production or application systems. In fungi, it is well-established that encapsulating conidia in biodegradable polymeric matrices can preserve their characteristics and enhance their effectiveness [88]; added to this, the physical barrier provided by encapsulation protects them from changes in pH, moisture, or ultraviolet radiation [89].

The techniques of spray-drying and ionic gelation (IG) stand out among those implemented for the encapsulation of microorganisms, with the former being less favorable, as, despite achieving successful encapsulations with live cells, it significantly decreases the maximum viability percentage during the manufacturing process [90]. Ionic gelation does not present these drawbacks due to the type of synthesis required and the biocompatibility of the materials used, which has been successfully explored in organisms such as *Beauveria bassiana* [88], arbuscular mycorrhizal spores [91], *Trichoderma asperellum* [92], *Trichoderma harzianum* [93–95], and *Trichoderma viride* [76,96].

It is important to remember that microencapsulation products derived by ionic gelation tend to have relatively low water permeability, although they contain a considerable amount of internal humidity [97]. This makes the particles a long-term method of delivering compounds, or in this case, biomass.

4.2. Reported Trichoderma Microencapsulation Models

Table 2 provides an overview of the encapsulated syntheses of *Trichoderma* sp. reported in the literature, along with some of their observed properties and characteristics.

Table 2. Properties of microparticles designed for biological control applications.

Type of Particle	Synthesis Method	Materials Used	Microorganism	State of the Inoculum	Applications	Country	Reference
Beads	Extrusion/ionic gelation	Montmorillonite, sodium alginate, glycerol, $CaCl_2$	*T. harzianum* (*UPM40*)	Conidial suspension grown on PDA medium	Agricultural applications as a delivery system for the biocontrol agent	Malaysia	[98]
Seed coating	Adhesion and drying	Sodium alginate	*T. viride*, *T. resei*	Not mentioned	Management of charcoal rot in sunflowers (*Helianthus Annuus* L.)	Pakistan	[99]
Microparticles	Ionic gelation	Sodium alginate	*T. reseei*	Not mentioned	Selección de esporas/mejoramiento genético.	Spain, Belgium	[100]
Beads	Droplets/ionic gelation	Sodium alginate, talc powder calcium gluconate	*T. viride*	Liquefied biomass and PDA culture medium	Enhanced growth of cabbage and red beet	Croatia	[101]
Microparticles	Water-in-oil emulsification/ionic gelation	Alginate, chitosan, peat, skim milk	*T. virens* (*TRS106*)	Conidial suspension grown on malt extract agar medium	Biological control of *F. oxysporum* wilt in tomatoes	Poland	[102]
Microcapsules	Ionic gelation/polyelectrolyte complexation	Sodium alginate, chitosan, copper sulfate pentahydrate	*T. viride*	Filtered biomass cultured in PDB liquid medium	Plant nutrition and protection	Croatia	[76]
Granules	Ionic gelation	Sodium alginate, soluble starch, citric pectin	*Trichoderma* sp.	Pulverized microorganism obtained from solid-state fermentation	Control biológico	Brazil	[89]
Microparticles	Ionic gelation	Sodium alginate, calcium chloride	*T. viride*	Filtered biomass cultured in PDB liquid medium	Agricultural applications as a delivery system of bioagent	Croatia	[103]
Microparticles	Ionic gelation/polyelectrolyte complexation	Sodium alginate, medium molecular weight chitosan, calcium chloride, eosin	*T. viride*	Filtered biomass cultured in PDB liquid medium	Agricultural applications as a delivery system of bioagent	Croatia	[104]
Microparticles	Ionic gelation	Sodium alginate, calcium chloride	*T. harzianum*	Pulverized microorganism obtained from solid-state fermentation	Biological control of *S. sclerotiorum* for applications in agriculture	Brazil	[95]
Beads	Ionic gelation	Sodium alginate, calcium chloride	*T. harzianum*	Conidial suspension grown on PDA medium	Biological control of *B. oryzaet* in rice	Malaysia	[105]
Beads	Extrusion/ionic gelation	Sodium alginate, montmorillonite, starch	*T. harzianum* (*UPMC243*)	Conidial suspension grown on PDA medium	Evaluation of conidia shelf life	Malaysia	[106]
Microcapsules	Spray drying	Maltodextrin DE20	*T. asperellum*	Pulverized microorganism obtained from solid-state fermentation	Agricultural applications as a delivery system of bioagent	Brazil	[107]
Beads	Ionic gelation	Cellulose nanocrystals (CNCs) and carboxymethyl cellulose (CMC), calcium chloride ($CaCl_2$)	*T. harzianum* (*LQC-99*)	Conidial suspension grown on PDA medium	Agricultural applications as a delivery system of bioagent	Brazil	[108]
Microcapsules	Ionic gelation	Chitosan, tripolyphosphate	*T. harzianum*	Extraction of fungi secondary metabolites	Biological control of *Macrophomina phaseolina* and growth promoter	Iran	[109]
Microparticles	Extrusion/ionic gelation	Sodium alginate (SA), calcium chloride ($CaCl_2$)	*T. asperellum*	Conidial suspension grown on PDA medium	Biocontrol of cucumber powdery mildew	China	[110]

Table 2. *Cont.*

Type of Particle	Synthesis Method	Materials Used	Microorganism	State of the Inoculum	Applications	Country	Reference
Microparticles/ microcapsules	Ionic gelation and spray drying	Maltodextrin, sodium alginate, carboxy methyl cellulose (CMC), gum arabic, gelatin, calcium chloride	*T. asperellum* (*TAIK 1*)	Not mentioned	Maintaining soil health, promoting plant growth, and reducing disease incidence by creating unfavorable conditions for pathogens	India	[111]
Beads	Ionic gelation	Alginate, Amidated pectinpectina amidada, calcium gluconate, plant based biochar, polydextrose	*T. koningiopsis* (*Th003*)	Conidial suspension grown on PDA medium	Biological control of sheath blight caused by *R. solani* in rice.	Colombia	[112]
Microparticles	Ionic gelation	Sodium alginate, calcium chloride	*T. harzianum* (*Ah90*)	Conidial suspension grown on PDA medium	Biological control of S. sclerotiorum and *Rhizoctonia solani* in tomatoes	Iran	[113]
Microcapsules	Layer-by-layer (LbL) encapsulation	Cationic lignin, lignosulfonate	*T. atroviride* (*TRS14*), *T. simmonsii* (*TRS75*), *T. gamsii TRS123*)	Not mentioned	Biological control of *Fusarium oxysporum* f. sp. *lycopersici* in tomato and improve spore stability	Germany and Netherlands	[114]

Regarding the cited papers in Table 2, the final size of the products is not the primary determinant in producing these types of bioinputs; rather, the focus is on improving or maintaining their effectiveness as biocontrol agents.

There is a notable preference for the use of ionic gelation (IG) as an encapsulation technique, with sodium alginate and calcium salts as the base of the formulations. This method is favored because it is a simple and efficient alternative for encapsulating molecules or structures of interest, requiring only an emulsion of the biopolymer mixed with the compound of interest and dispensed into another solution containing a divalent cation [115]. Additionally, this technique can be complemented with drip or extrusion technologies to form uniformly sized particles, which, while improving the applicability and standardization of the formulation, also involve associated production costs.

For all scenarios, the particles created with these technologies are typically made from food-grade biopolymers based on proteins or polysaccharides, including pectin, chitosan, collagen, gelatin, and the most popular alternative, alginate [77,116]. These confer a high degree of biodegradability and environmental integration, in addition to being produced through green chemistry. This allows their incorporation into applications for human consumption, agricultural production, or the pharmaceutical industry, among many other areas.

Specifically, alginate (ALG) is an excellent biocompatible alternative for encapsulation since it is a low-cost linear anionic polysaccharide obtained from multiple species of algae. It consists of glycosidic (1,4) bonds of β-D-mannuronic acid (ManA) and α-L-guluronic acid (GulA), where the molar ratio of each monomer depends on the raw material's source and its processing [117]. For instance, capsules acquire different properties of elasticity and strength depending on the proportion of GulA, with higher concentrations leading to greater rigidity in their molecular structure compared to chains predominated by ManA [118].

Another feature that contributes to the differences in the proportion of GulA residues is the affinity to form cross-links with divalent ions such as Zn^{2+}, Ca^{2+}, or Ba^{2+} or with trivalent ions in a composite structure that forms gels through the cooperation of several ALG chains commonly known as the "egg-box" model [118]. This can be leveraged to work with different elements, thereby granting desirable properties to the particles, for example, as a protective product and as a biofertilizer [119]. Not to mention the natural chelating

properties of alginate in the chelation of ions like Cu^{2+} or Zn^{2+} [78] and its ability to retain soil moisture [120].

This analysis provides evidence that although it is a widely used technology, the combination with different ions or integration with other polymers through ionic complexations has not been fully exploited and could be a strong point for research.

Another notable finding shown in Figure 3 is that, according to our search, most of the reported formulations were developed in Brazil, Croatia, and Malaysia—countries that, except for Croatia, have high levels of biodiversity and tropical climates. Central Europe had the most references on the topic; however, this research trend does not seem to have been significantly adopted in regions with temperate climates or first-world economies, such as countries in North America or Western Europe. Furthermore, Central and Southern Asia have shown interest in this topic in recent years, and this trend may increase.

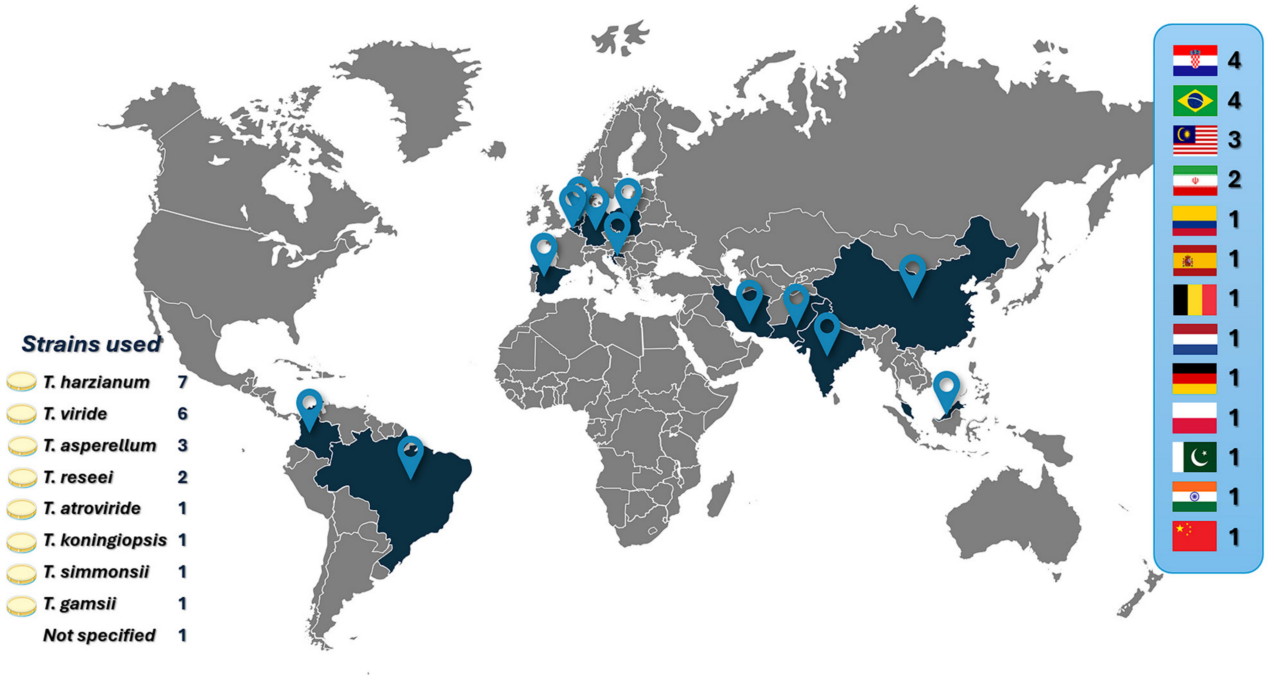


Figure 3. Distribution of *Trichoderma* strain encapsulation efforts in the world.

For Central America and Africa, developing encapsulation technologies with polymers could be an attractive alternative offering competitive advantages to their markets, where the economy is predominantly agricultural. It also opens the window to explore the biodiversity in the area and address production issues through specific approaches for each locality and its crops.

We did not find quantified data on the economic and commercial impact that *Trichoderma* encapsulates currently have within the BCA market. However, this category of agricultural products has had a growing overall trend due to international regulations on organic production [121].

According to a report from 2022 [122], the BCA market grew by 60% between 2012 and 2020, reaching a value of $3 billion, which represented approximately 5% of the global crop protection product market at that time ($62 billion). This growth was significantly higher compared to conventional chemical-based agricultural products, which grew by only 14% during the same period. It also indicated that by 2022, there were approximately 1000 active ingredients in the BCA market worldwide, marketed in mixture or pure forms through about 5000 different brands promoted by around 500 companies, with average annual sales of $10 million per company [122]. In 2023, the total value was around $5.2 billion, and the forecast proposes a compound annual growth rate (CAGR) between 2024 and 2032 of over 16.4% [121].

Considering the distribution of companies within this market, most commercial activity was registered in Europe (31%), North America (29%), and Asia (24%), followed by Latin America (15%) and finally, the Middle East and Africa (1%). In terms of BCA usage, most products are indicated as bioinsecticides (70%), biofungicides (25%), bionematicides (3%), or bioherbicides (2%). Between 70 and 80% of applications are concentrated on fruit, vegetable, flower, and ornamental crops [122].

Products based on the genus *Trichoderma* represent nearly 50% of the fungal BCAs in the market, with a notable preference for the *harzianum*, *viride*, *asperellum*, *atroviride*, and *koningii* strains [30,123]. Similar occurrence is also observed in encapsulation studies for the mentioned strains (Figure 3), suggesting that the application of technology is driven more by market demands than by microbial resistance or process compatibility.

The three most popular strains that we have identified as leading the efforts are:

- *T. harzianum*: Known for inducing systemic resistance in plants through the production of enzymes and metabolites that stimulate plant immune responses and enhance growth. It is also effective in environments where chemical fungicides are ineffective due to its ability to efficiently colonize plant roots and persist in the soil [124].
- *T. viride*: Produces extracellular enzymes, such as cellulases and chitinases, that degrade the cell walls of plant pathogens, inhibiting their growth and recycling nutrients in the soil. This enhances soil fertility and makes it an effective biocontrol agent against root rot and other soil-borne diseases [125].
- *T. asperellum*: Exhibits biocontrol activity by competing for space and nutrients, suppressing the growth of fungi. It promotes plant and root growth, enhancing nutrient uptake and improving the soil microenvironment [123].

However, their selection may also be influenced by their similar action at the soil level.

There are multiple examples of BCAs produced with *Trichoderma* that have been validated under real conditions. The majority are traditional formulations whose characteristics have been mentioned throughout this manuscript. A representative list of commercial products/trademarks is summarized in the review of Ghazanfar et al., including more than 40 examples for readers to check [125]. According to the evidence presented in Table 2, *Trichoderma* microencapsulation formulations currently appear to still be in the development stage, as the available documentation is more focused on academic research than on commercial production. However, many of the cited studies have demonstrated the effectiveness and advantages of microencapsulated *Trichoderma* both in vitro and in field conditions, and they show promise for scalability in short- and medium-term production.

5. Conclusions

The technification of traditional biocontrol methods through the application of encapsulation technologies to biocontrol agent (BCA)-based products can offer highly valuable alternatives in agriculture and the market. Integrating fungal structures within biopolymer matrices can significantly enhance the natural dispersion and resistance capabilities of organisms such as *Trichoderma*.

According to this review, structuring through ionic gelation stands out in these applications due to its simplicity and high versatility in creating various products with specialized or multifunctional characteristics. Biodegradable polymers like alginate are suitable for use as raw materials, which, in addition to being combinable with ions that act as nutrients in plant species, do not require a high economic investment to obtain the necessary materials.

Multidisciplinary research utilizing micro-, nano-, and biotechnology offers a great alternative to address the challenges currently faced by agriculture, promoting the transition to green technologies that are compatible with soil diversity and sustainable development. The ability to address problems with specificity on a small scale can be a key element in modeling current and future agricultural scenarios.

Author Contributions: Conceptualization, E.V.-R. and R.C.-C.; writing—original draft preparation, E.V.-R. and R.C.-C.; writing—review and editing, E.V.-R., R.C.-C. and W.R.-M.; visualization, E.V.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was founded by the Vice-presidency of Research and Extensión. Costa Rica Institute of Technology. Project: Development of a microencapsulated prototype of the biocontrol fungus Trichoderma harzianum obtained from conidia fermentations in a bioreactor. Program N°4 -Research. Project 49-2022-65-2023.

Data Availability Statement: Not applicable.

Acknowledgments: Icons used in this review by Servier, with some modifications in color, size, or combination of the original images or correction of minor details. https://smart.servier.com/ (accessed on 20 May 2024) is licensed under CC-BY 4.0. Unported https://creativecommons.org/licenses/by/4.0/ (accessed on 20 May 2024).

Conflicts of Interest: The authors declare no conflicts of interest.

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