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Review Article

Harnessing Fungal Power: The Development and Formulation of Agricultural Bioinputs

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Abstract

The excessive use of chemical pesticides in agriculture harms the environment and health and promotes pest resistance. Biopesticides, such as mycopesticides, offer more sustainable alternatives to combat plant diseases. Entomopathogenic fungi have been used as mycopesticides for the biological control of pests, with host specificity reducing non-target risks. In addition to their entomopathogenic activity, these fungi also can promote plant growth by inducing resistance to diseases, forming endophytic relationships with plants, improving nutrient transportation and productivity, and interacting with other soil microorganisms, improving their conditioning. The effectiveness of mycopesticides can be compromised by the selection of strains, the appropriate production process for the microorganism, the stability of the product, compatibility with other chemical products, as well as instability with environmental conditions. The selection of resistant propagules during production is fundamental for the creation of stable and effective mycopesticides. Regulatory issues and farmer adherence to bioinputs are also impacting factors in the process. This review describes the role of entomopathogenic fungi in agriculture as a bioinput, paying special attention to their characteristics, mechanisms of action, mode of production, environmental and economic impact, as well as the implications of technological advances in the development of these products.

Keywords: Entomopathogenic Fungi; Mycopesticides; Biological Control; Agriculture; Sustainability

Abbreviations

AMF: Arbuscular Mycorrhizal Fungi; BPPD: Biopesticides and Pollution Prevention Division; EPF: Entomopathogenic Fungi; IPM: Integrated Pest Management; ISR: Induced Systemic Resistance; MAPA: Ministério da Agricultura, Pecuária e Abastecimento; MEOR: Microbial Enhanced Oil Recovery; NBP: National Bioinputs Program; PRIA: Pesticide Registration Improvement Act; SAR: Acquired Systemic Resistance; SSF: Solid State Fermentation;

Introduction

Commercial synthetic insecticides are widely used to reduce the density of pests in crops. However, these agricultural chemicals have negative impacts on the agroecosystem, affecting crop productivity, harming human health and causing damage to non-target beneficial organisms. In addition, the repeated use of pesticides from the same group leads to the development of resistance and the resurgence of insect pest populations [21,25]. As a result, efforts have been made to adopt alternative and ecologically sustainable strategies to protect crops in the long term and preserve the environment. Biopesticides have stood out as a viable option due to their environmentally friendly, safe properties, high efficacy and rapid decomposition. Microbiome-derived biopesticides offer a non-toxic approach to controlling insect pest populations, contributing to improving the quality and quantity of agricultural products. This is essential given the need to minimize the adverse effects of traditional insecticides [21].

Biological control of insect pests with entomopathogenic fungi (EPF) is an effective and desirable strategy that involves the use of natural microorganisms to suppress the activity of these pests, offering an alternative to chemical insecticides. These EPF exhibit important biological characteristics, such as selectivity to the target, high reproductive rate, rapid life cycle and long survival, fundamental aspects in the biological control of insect pests [36].

Mycopesticides are pesticides whose main active ingredient is a fungus. They are divided into several types. The current formulations of biopesticides available on the market, in the form of powder, granules and oil. Developing an effective formulation is essential for the successful use of commercially available biopesticides. Several factors impact commercial formulations, such as shelf life, biological and physical characteristics of the formulation. It is highly desirable that the effectiveness of the formulation is maintained throughout the main growing seasons and ideally for an even longer period. In this way, a formulation with an extended shelf life is considered valuable for both manufacturers and end users [3].

Therefore, this review addresses the use of EPF in agriculture, as well as their functions, establishing key points in their formulation and production, in addition to identifying the challenges and limitations for the use of these bioinputs in a more sustainable environment.

Use of entomopathogenic fungi in agriculture

The EPF are crucial to Integrated Pest Management (IPM) globally. EPF are eukaryotic, heterotrophic microorganisms that can be unicellular or multicellular (filamentous). They reproduce sexually and asexually, generating various infectious propagules. The effectiveness of EPF in the field might be influenced by environmental conditions, such as exposure to UV light, temperature variations and humidity [5].

The orders *Hypocreales, Onygenales* (specifically the genus *Ascosphaera), Entomophthorales*, and *Neozygitales* are known to harbor a wide diversity of EPF [38]. These fungi have a broad range of hosts and exhibit varying toxicity levels, as well as the ability to suppress chewing and sucking insect pests. As a result, they have gained a significant role as biocontrol agents. Entomopathogenic microbiomes are generally notable for being more environmentally friendly, specific to their applications, suitable, and cost-effective [19].

The order Hypocreales is a diverse group of fungi belonging to the class Sordariomycetes within the division Ascomycota. This order includes a wide range of fungi with various ecological roles, including plant pathogens, saprophytes, and entomopathogens. Hypocreales is characterized by the production of perithecial ascomata, which are specialized fruiting structures that contain sexual spores called ascospores [38]. Many members of the Hypocreales order are important in agriculture, forestry, and medicine. They are known for their ability to produce a variety of bioactive compounds, including enzymes, secondary metabolites, and mycotoxins. Some are used as biological control agents to manage agricultural pests, while others are responsible for plant diseases [20,38]. One notable group within Hypocreales is the entomopathogenic fungi, which are specialized in infecting and killing insects. These fungi have gained attention as environmentally friendly alternatives to chemical insecticides in pest management strategies, playing a crucial role in maintaining pest control efficacy, reducing the risk of resistance to chemical pesticides, and promoting environmentally sustainable pest suppression. The genera *Beauveria*, *Hirsutella*, *Isaria*, *Metarhizium*, *Cordyceps*, *Trichoderma*, and *Purpureocillium* are some examples of entomopathogenic fungi in this order [20].

The Eurotiales order also includes various filamentous fungi such as the genus *Aspergillus*, *Penicillium*, *Talaromyces* and *Paecilomyces*. *Paecilomyces* is important in food production, medical research and agricultural biotechnology. This genus is known to include species with diverse applications, including the control of agricultural pests to the production of industrial enzymes [20].

Entomopathogenic fungi action mechanisms

Entomopathogenic mechanisms

The EPF as mycopesticidas can be applied in three broad approaches to biological pest control: classical biological control, augmentation and conservation. In classical biological control, a control agent is used to effectively combat the population of harmful insects, often involving the introduction of an exotic agent to establish long-term sustainable and economical control in a new environment. On the other hand, biological control by inoculation involves the deliberate and controlled release of small quantities of fungal biological control agents, which multiply and keep the pest population under control for an extended period. Although these natural enemies are not capable of permanently eradicating a high pest population density, they can keep it at acceptable levels. However, they require periodic re-inoculations to maintain their effectiveness. Finally, biological control by flooding involves the massive release of natural enemies for immediate and short-term pest population control. The released natural enemies themselves carry out population control, resulting in a rapid decrease in both pests and natural enemies over time. This approach is associated with the use of mycopesticides [5].

The term "mycopesticide" refers to a biological pesticide that uses entomopathogenic fungi to combat insect pests. These microorganisms can selectively infect and kill insects, offering a more sustainable and environmentally friendly alternative to traditional chemical pesticides. Hyphomycetes (Deuteromycetes) is one of the classes of fungi which are used as biopesticide in worst part of the world. Their conidial structure and host range is different from another fungal group [25].

In nature, EPFs play a fundamental role in the population control of insects and mites. Their host specificity significantly reduces the risk of affecting non-target species. These fungi produce spores (including conidia and blastospores), which initiate infection by germinating on the host's surface and then spreading throughout the body via the outer cuticle [5]. The infection process goes through several stages, including the attachment of the spores to the insect's cuticle, the penetration of the germ tube into the cuticle, the development of the fungus inside the insect's body and the colonization of the fungal hyphae in the hemocele. The presence of a mucous layer of proteins and glucans on the spores facilitates their adherence to the insect's cuticle, forming specialized structures called appressoria during germination. Mechanical pressure and enzymatic activity, such as lipases, play a crucial role in this process. Generally, EPFs develop in the insect's hemocele, and death occurs due to mechanical damage caused by fungal growth inside the insect (mummification) or the action of poisons released by the pathogen [32]. During the pathogenic phase, various mycotoxins, such as Beauvericin (*B. bassiana*), Beauverolides (*Verticillium lecanii*), Bassianolide (*Paecilomyces spp.*) and Destruxins A, B, C, D, E, F (*M. anisopliae*), are produced and are toxic to insects. After the death of the insect, the fungus generates hundreds of new spores in the corpse, which spread and continue the life cycle of the fungus, looking for new hosts to infect. The fungal species and the number of spores is responsible for the infection rate [32].

Several hypocrealean fungi, such as *I. fumosorosea,* B. *bassiana*, *H. thompsonii*, *L. lecanii*, *Metarhizium acridum*, *M. anisopliae* and M. *brunneum*, are commercially available worldwide as biopesticides, offering a variety of formulations. These biopesticides have proven efficacy against pests that have different types of mouth apparatus, both piercing and sucking and chewing. What makes these entomopathogenic fungi remarkable is their ability to colonize the tissues of host insects without the need for lethal toxins [6].

Non- entomopathogenic mechanisms

Although the entomopathogenic role played by these fungi is widely recognized, recent studies have significantly advanced the understanding of EPF by exploring their interaction with soil, plants and other microorganisms in the environment. These non-pesticide-related characteristics play a crucial role in the adaptation of fungi, enabling them to survive in soil and plant environments, even in the absence of a host arthropod [6].

EPFs are capable of inducing plant resistance by influencing the production of defense proteins and gene expression. Plants have two types of induced resistance: acquired systemic resistance (SAR) and induced systemic resistance (ISR), as defenses against biotic and abiotic stressors. SAR is triggered by salicylic acid and pathogenesis-related proteins when exposed to pathogens, while ISR is activated by non-pathogenic microbes, involving the jasmonic acid and ethylene pathways. Both pathways lead to the production of defense proteins and enzymes. EPF, combined with other microorganisms, such as *Pseudomonas fluorescens*, increase the accumulation of defense enzymes, protecting against pests and diseases and promoting plant growth [35].

Beauveria bassiana and other hypocreales fungi form endophytic relationships with various plant species. They colonize plants when applied by different methods and provide benefits to the plants. In return, these fungi can survive around plants without arthropod hosts and obtain nutrition from them. Other studies have shown that *B. bassiana* and related fungi improve the productivity of green beans, soybeans, switchgrass and wheat by translocating insect-derived nitrogen. Symbiotic soil fungi and possibly entomopathogenic fungi improve nutrient transport mechanisms in plants for various soil nutrients. In addition, plants exchange photosynthates for insect-derived nitrogen with the fungus. This research provides valuable information on the complex relationships between fungi, insects and plants [8].

In strawberry fields, soil application of *B. bassiana, M. brunneum* and I. *fumosorosea* had a positive impact on fruit production, with increases ranging from 3.5% to 9.6%. In addition, foliar spraying of *B. bassiana* and *I. fumosorosea* resulted in a 14.4% and 4.4% higher fruit yield than the untreated control in strawberry plots. In a Chinese study, seed treatment with *B. bassiana*, *I. fumosorosea* and *Leacanicillium lecanii* improved plant height and fresh biomass of green beans, with a *B. bassiana* isolate standing out as the most effective in promoting plant growth [11].

The mechanisms of disease antagonism include competition for space, parasitism and induced systemic resistance. EPF, especially *B. bassiana*, have shown significant protection against plant diseases, even outperforming botanical and microbial fungicides in some cases. Studies have also shown that EPF can improve crop health in the presence of pathogens such as *Macrophomina phaseolina* in strawberries [34].

EPFs can exert a positive influence on plant health by antagonizing plant pathogens through parasitism, competition, antibiosis or the induction of systemic resistance in plants. Competition is the unequal behavior of two or more organisms for the same requirement or resource, so that its use by one of them reduces the amount available to the others. These requirements or resources can be nutrients, oxygen, physical space, light, etc. Studies have shown that *Beauveria spp*. has antagonistic effects against various plant pathogens, including *Botrytis cinerea, Fusarium oxysporum*, *Gaeumannomyces graminis*, *Pythium sp*, *Rhizoctonia solani* and *Sptoria sp*. In addition, *B. bassiana* was effective in protecting cotton and tomatoes against *Rhizoctonia solani* and *Pythium myriotylum* through seed treatments and against *Xanthomonas axonopodis pv. malvacearum* in cotton through soil applications. These treatments resulted in increased plant growth and reduced disease severity [34].

Trichoderma is an important plant growth promoter, stimulating both the development of deep and vigorous roots, making plants more resistant to drought, and the absorption and solubilization of nutrients. Its ubiquity in natural and agricultural soils, its ability to decompose organic matter and its metabolic versatility are evidence of its effective competition for resources. Successful colonization of the rhizosphere is crucial to its success, allowing it to produce siderophores and solubilize phosphates. In addition, its remarkable tolerance to oxidative stress makes *Trichoderma* capable of surviving in environments with high levels of ROS (reactive oxygen species), differentiating it from other fungi [34].

The process of adherence to the root surface in *Trichoderma* may be mediated by small hydrophobic proteins on the outer surface of the cell wall (hydrophobins) and by expansin-type proteins capable of recognizing cellulose and modifying the architecture of the plant root. The establishment of *Trichoderma* in root tissues can be favored by hemicellulase and polygalacturonase activities, the latter required by *T. harzianum* for active colonization of the root and the induction of plant defenses against the attack of a necrotrophic pathogen such as Botrytis cinerea [24,34].

Key points in the formulation of fungus-based bioinputs

The application of plant beneficial microorganisms has been widely accepted as an efficient alternative to chemical fertilizers and pesticides. Isolation and selection of efficient microorganisms, their characterization and testing in soil-plant systems are well studied. However, the production stage and formulation of the final products are not in the focus of the research, which affects the achievement of stable and consistent results in the field [43].

Biotechnology offers a wide array of techniques leading to bioformulates: starting from selection of promising microbial strains, characterizing their morphological, physiological and biochemical properties, testing their activity under fermentation and soil conditions and, finally, formulating them into commercial products [22]. Recent analysis of the field of plant beneficial microorganisms suggests a more integrated view on soil inoculants with a special emphasis on the inoculant production process, including fermentation, formulation, processes, and additives. In this section we will be focusing on the production process [43]. One of the most dynamic and expanding fields of research during the last years is the production, formulation and application of biofertilizers and biocontrol agents. Here, we will present examples of the effect of different types of fermentation processes on the behavior, but also on biocontrol agents [17].

Bearing in mind the key role of soil microorganisms in sustainable agriculture and the concerns regarding crop quality and human health, as well as the problems of a growing human population in combination with climate change, the biotechnological approach in production and formulation of plant beneficial microorganisms should be in the focus of the scientific community. Independently of the mode of fermentation (submerged or solid-state) or cell state (free or immobilized), we can always improve the process productivity by optimizing the initial pH, agitation rate, aeration volume, initial inoculum concentration, and (in SSF) initial moisture and type of the moistening agent [17]. The assessment of the effect of different fermentation parameters on the biomass accumulation and cell survival in the bioformulates during storage is essential to the development of stable commercial products. The optimization schemes and experiments are normally carried out in controlled conditions simultaneously or by analyzing the effect of one parameter (variable) at a time, independently of the mode of fermentation (solid, liquid, free/immobilized cells, etc.) [18].

The initial medium pH is important for the adaptation of the inoculum, affecting microbial growth, and, later, its metabolic activity and production of enzymes, antibiotics, organic acids, phytohormones and biocontrol potential as well. Microbial growth is strongly dependent by the pH as it affects the surface of cells and increases nutrient absorption. Most microorganisms are reported to prefer neutral and slightly acidic pH with optimal biomass production in the range of 4.5 and 6.5. A pH value below 4 or above 7.0 slows fungal growth [18]. Some species were found to tolerate alkaline pH while others, mainly some organic acid producers, preferred an acid environment. Other acid producers, however, need higher pH and the presence of a neutralization agent ($CaCO_3$ or NaOH, $Ca(OH)_2$) to produce the acid [16]. Many filamentous fungi, including some *Trichoderma spp.*, decrease the medium pH during their growth, but after assimilation of the available carbohydrate substrate the opposite pH tendency is observed which influenced the biomass growth and enzyme production [18]. These details are important when the aim of the fermentation is to solubilize insoluble inorganic phosphate to enrich the final liquid with plant available P, to produce enzymes (for example cellulase) in the fermentation broth or when a bio-formulate is prepared for a soil-plant system to enhance the plant available P or degrade cellulose-bearing wastes [22].

Temperature is another important fermentation process parameter. There are plant beneficial microorganisms sensitive (or not) to high or low temperature but, in general, high temperatures facilitate the biomass growth. It was suggested that as the soil microbial community composition is affected by the climate change and freeze-thaw cycles, the effect of the temperature should be studied more in detail [17]. Formulated inoculants can be produced by air-drying, desiccation, lyophilization, and spray drying, among others Lyophilization (freeze-drying) is a soft dehydration method, which preserves the cell viability of the bio-formulations. It was considered that fermentation pH and temperature affect the cell stress resilience and efficacy of this method. Optimization of the fermentation parameters can be carried out including in the optimization scheme components of the medium [44].

The main processes for biomass/spores production of plant beneficial microorganisms are based on aerobic fermentations. In case of submerged fermentations particularly important is the dissolved oxygen as it is the main parameter for successful microbial growth, which directly affects metabolic activity and final products type [17]. This phenomenon is more pronounced, specifically in fed-batch operations when the biomass concentration is varying. At laboratory conditions, the level of dissolved oxygen can be manipulated by the shaker speed, while using fermenters the oxygen dissolved in the medium is controlled by manipulating the airflow or the stirrer speed [22]. Applying different configurations of flow impellers plays an additional role in the dissolved oxygen level and affects cell morphology, mass transfer, medium viscosity and rheology, which improved the product formation. In solid-state fermentation, the use of special cultivation bags ensures gas exchange or alternatively, special designed bioreactors and oxygen-enriched air could be supplied, thus providing sufficient oxygenation necessary for optimal biomass accumulation and sporulation on solid-substrate fermentations [33]. Initial moisture and water activity, the nature and size of the solid particles, inoculum volume, and additional nutrients also affect the efficacy of the solid-state fermentation [42]. All these factors must be optimized applying strategies, which include factorial design and response surface methodologies, artificial neural network and genetic algorithm [17]. In some cases, the optimization strategies for fermentation parameters are oriented to shape the cell morphology [16]. If the aim of the fermentation is to produce an efficient, multifunctional product with high level of both biomass and metabolites, it shall be considered that the metabolic productivity is particularly dependent on an optimal morphology (free cells of pellets): another important critical parameter specially for fungal or filamentous microorganisms [30].

The selection of the fermentation mode can be determined experimentally as microbial biomass and spores from plant beneficial microorganisms can be produced by both submerged and solid-state fermentations. The first process, either as a single batch or fed-batch, is the most effective and accepted method to produce biomass, spores, and metabolites on an industrial scale. On the other hand, the second one is instead a relatively well-established fermentation technology to produce various metabolic products and significant amount of biomass and spores [22].

Mycorrhizas are also involved in biological processes, which improve plant health, through increased protection against biotic and abiotic stresses, improve soil quality and structure, and enhance nutraceutical value of horticultural products. The most important advantage of using arbuscular mycorrhizal fungi (AMF) as biostimulants is related with their role in facilitating phosphate and micronutrients uptake by plants. While almost all plant beneficial microorganisms including some ectomycorrhizal and mycorrhiza-like can be produced by cultivation in fermentations systems, AMF cannot be mass-produced without plants [1]. The requirements for development and selection of bio-techniques for mass production of mycorrhiza are like those of other microbial fertilizers. The final product should be free of pathogens, viable after periods of storage, with a high colonization power and easy to apply [40]. The main problem in the case of AMF is the lack of both free-of host efficient production methods and formulations that cannot ensure high final product quality [1]. AMF are commonly produced in scaled-up pot plant-based culture using sterilized substrates. However, this method is not economically acceptable in industrial conditions. Soilless, hydroponic culture is another mode of AMF production as it provides high quality inoculum with higher spore number compared to the pot cultivation in greenhouse [27].

Sourcing of bioinputs: critical factors in formulation

For the formulation of commercial biological products, it is important to pay attention to important points such as the selection of strains, the production process suitable for the microorganism, the stability of the product as well as the compatibility with other chemicals [39].

In the development of new biocontrol products, the production of fungal propagules can be achieved by two main mass production methods: solid and submerged fermentations. In the former, a nutrient-poor substrate, usually with low water activity, is used to sustain growth at the interface with the atmosphere, allowing an abundant supply of oxygen for sporulation, resulting in aerial conidiophores and conidia. These asexual propagules consist of important fungal structures for dissemination and infection [15]. This method is easily carried out with low-cost equipment. The major obstacle to this production lies in the time required to produce aerial conidia. Generally, achieving a high spore yield with this fermentation process takes 10-15 days or even longer, depending on the fungal species and isolate. At the same time, it also depends on large growth rooms with controlled environmental conditions, such as photoperiod and temperature [33]. There are problems in controlling fermentation parameters such as pH, water activity, aeration and nutrient levels during fungal growth, while the risk of contamination may be more frequent due to lack of automation correcting deficiencies of carrier-based biofertilisers [4].

In case of the chemical products used to formulate bioinputs, the correct use and selection of surfactants is a key point in the formulation. Biosurfactants are created by microorganisms and have numerous distinguishing qualities when compared to other synthetic surfactants, such as moderate manufacturing conditions, multifunctionality, better biodegradability, and reduced toxicity of live cell synthesis of active chemicals [7]. Biosurfactants are produced on the microbial membrane or excreted over the outer membrane and have both hydrophilic and hydrophobic areas. Hence, biosurfactants are used in the medical industry, bioremediation, microbial enhanced oil recovery (MEOR), and the pharmaceutical industry [12]. In the food business, biosurfactants are frequently employed as anti-adhesive agents, emulsifiers, de-emulsifiers, spreading agents, foaming agents, and detergents that have applications in a variety of industries, including agriculture, the industrial sector, and the environmental area. Oil zapper is the most recent bioremediation application [31].

Biosurfactants are potential candidates for future crop protection applications due to their diverse functionalities and participation in biological control by acting as either antifungal agents or inducers of induced systemic resistance. In terms of biological control, biosurfactants often work by disrupting the pathogen's cell surface and creating channels in the cell wall. From among the various kinds of biosurfactants, glycolipids, such as cellobiose lipids and rhamnolipids, and cyclic lipopeptides, such as surfactin, iturin, and fengycin, protect plants through their antifungal activities against phytopathogenic fungi [7]. The possible use of biosurfactants as biological control agents was addressed by Stanghellini and Miller in 1997 [37] they explain how rhamnolipids can break zoospore membranes and induce lysis of zoospores of several oomycete plant diseases. Since then, several papers have covered the significant function of rhamnolipids in the defense against different phytopathogenic fungi. By preventing *B. cinerea's* mycelial development and spore germination, rhamnolipid biosurfactants were discovered to exhibit direct antifungal effects. The main component of di-rhamnolipid in this cell-free medium, which is distinguished by better lysis traits over mono-rhamnolipid to rupture the spore membranes, could be responsible for the cell free medium's significantly better antifungal effectiveness [37]. This is especially true for plant pathogens that produce zoospores. Similar to rhamnolipids, cyclic lipopeptides are a type of biosurfactant that have antifungal efficacy against phytopathogenic fungi. The method of hydrophilization employing biosurfactants results in good wettability, suppression of pesticide toxicants, and uniform dispersion of fertilizers in the soil [12].

Nanotechnology based biofertilizer has the potential to revolutionize the agricultural systems and numerous other areas. Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 and 100 nm, which can drastically modify their physicochemical properties compared to the bulk materials. Due to its high surface area to volume size ratio, they exhibit significantly novel and improved physical, chemical and biological properties, phenomena and functions. Nanotechnology based biofertilizer as bio-tech innovations; it is the matter at nanoscale (1 – 100 nm) dimensions. Biomaterials when reduced to the nanoscale show some properties which are different from what they exhibit on a macro scale, enabling unique applications. To synthesis of nanonutrients, microorganism was grown over selected nutrient source and provides necessary growth conditions. After the complete growth the biomass was separated. The filtrate was used for isolation of extracellular specific proteins, and these were used for nanoparticle synthesis [4]. The selection of microorganism and optimum parameter are specific for synthesis for desired type of nanonutrients. Through catalytic effects, microbial extracellular secreting enzymes could produce reducing the metal salt of macro or micro scale into nano-scale diameter. These nanoparticles get into plant cells through either stomatal or vascular system which may enhance plant cell metabolic activities that lead to higher crop production. It is suggested that the stomatal pathway is highly capacitive because of its large size exclusion limit and its high transport velocity [28]. Such biologically synthesized, very tiny functional nanoparticles are economically chief, relatively stable, easy downstream processing and environmentally safe as they are encapsulated by fungal protein which is water soluble. In general, the synthesis of bio- and nano-fertilizers could be achieved using microorganisms, where some nanofertilizers may result from the biological method. Furthermore, the synthesis of nanoparticles using biological system is in wide research due to the potential applications in nanomedicine. The biological synthesis of nanoparticles is less expensive and eco-friendly [31]. As mentioned before, the synthesis of nanoparticles could be mainly achieved through the physical, chemical and biological methods. Concerning the biological method, it could be produced nanoparticles from reduction and oxidation processes from small entities using lesser defects in-vitro or in-vivo. Several substances mainly could be used as reducing and stabilizing agents during this synthesis process including proteins, enzymes, sugars and phytochemicals such as phenolics, flavonoids, cofactors, terpenoids, etc. It is reported that, some nanoparticles could be used in nanofertilization, which generated through the biosynthesis process in many studies [28].

Therefore, it could be concluded that, biosynthesis of nanofertilizers could be achieved through many microorganisms and plant extracts. Concerning the biological method for nanofertilizer biosynthesis, it could be produced nanoparticles through many biotech innovations [31].

Novel biotechnology techniques in the preparation of agricultural bioinputs

The application of biosynthesized nanoparticles in agricultural sector may lead to sustainable development. Hence, this leads to sustainable agriculture through putting less inputs and generating less wastes, minimizing nutrient losses, and release nutrients at a proper rate for plant demand comparing with conventional farming [4].

The live microorganisms could be delivered through the carrier. It could be defined the carrier as an inert material using in transporting microbes from factory or laboratory to soil [28]. These carriers in general should be characterized with certain properties and superior-quality carrier materials for microbial inoculants include: (1) The carrier should be the major portion of the inoculant to help in delivering the suitable amounts of microbes in a good physiological condition, (2) It should be designed to provide a suitable microenvironment for the microbes, easily biodegradable, nontoxic and nonpolluting, (3) It should be stable at room temperature or it has a sufficient shelf life nearly at least 2-3 months, (4) It should be in a good moisture absorption capacity or high water-holding and water retention capacity as well as suitable for almost bacteria (5) Easy to sterilize by autoclaving or other methods, (6) Low cost, available in adequate amounts and good pH buffering capacity, and (7) Carrier of inoculants should be proper for surviving the microbes. It could be prepared the formulation into two methods including mixture the inoculum with solid and liquid carriers. Concerning solid carrier materials, they have advantages in increasing the supply of nutrients like phosphorus to plants, biological degradation of organic pollutants and resistance to soil-borne plant pathogens. Many inorganic substances and organic carriers have been used as carriers including talc formulation, press mud formulation, vermiculite formulation, alginate beads, biochar, perlite and peat formulations [4]. Furthermore, it is reported that, each gram of carrier of products should contain at least 10 million viable cells of a specific strain [28]. On the other hand, many advantages of liquid inoculants have been reported to include no need for any sticker materials, a less amount of inoculant is needed, high number of cells will be supported for a long time, easy to produce, sterilize completely preventing contamination, a large number of inoculums could be transported in small bottles, applying as fertigation, compatible with modern agriculture machineries, could be used for stress alleviation [39]. Carriers could be divided into the following categories: (1) soils (likeclays, peat, coal and inorganic soil), (2) plant waste materials (such as farmyard manure, composts, soybean meal, soybean, peanut oil, wheat bran and press mud), (3) inert materials (like perlite, vermiculite, ground rock phosphate, calcium sulfate, poly-acryl-amide gels and alginate beads), (4) plain lyophilized microbial cultures and oil dried bacteria, (5) liquid carriers(like broth, broth + polyvinyl-pyrrolidone) and (6)capsule-based carriers such as pelleted spores and cells in capsules [4].

Nanotechnology has the potential to revolutionize the agricultural systems and numerous other areas. Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 and 100 nm, which can drastically modify their physicochemical properties compared to the bulk materials. Due to its high surface area to volume size ratio, they exhibit significantly novel and improved physical, chemical and biological properties, phenomena and functions [4]. Nanotechnology as bio-tech innovations; it is the matter at nano scale dimensions. Biomaterials when reduced to the nanoscale show some properties which are different from what they exhibit on a macro scale, enabling unique applications [41]. Through catalytic effects, microbial extracellular secreting enzymes could produce reducing the metal salt of macro or micro scale into Nano-scale diameter [15]. In general, the synthesis of bio- and nanobiofertilizers could be achieved using microorganisms, where some nanofertilizers may result from the biological method. Furthermore, the biological synthesis of nanoparticles is less expensive and eco-friend [4].

Limiting factors of mycopesticide development

The excessive use of phytosanitary products, such as chemical pesticides, in agriculture impacts the environment, causing soil and groundwater pollution, harming beneficial insects and promoting resistant pests, as well as having deleterious effects on farmers' health. This has led to growing pressure to adopt ecological alternatives, such as biopesticides, to protect crops. Biopesticides, including mycopesticides, are used to combat plant diseases in a sustainable way by inhibiting pathogens and strengthening plant resistance. EPF-based mycopesticides have proven to be promising alternatives to traditional chemical pesticides due to their selectivity and lower environmental impact. However, they also face some limitations that may affect their development and widespread adoption [13].

A competitive mycopesticide is characterized by a number of qualities, such as: performance on a par with corresponding chemical pesticides or the ability to be integrated into IPM programs, preservation of product quality throughout transport and storage, biodegradability, a lower level of toxicity and impact on the environment compared to chemical pesticides, ease of production on a large scale, simplicity of application, compatibility with agronomic equipment and practices, a viable cost/benefit ratio and the possibility of registration. Any lack of one of these characteristics can compromise the commercial potential of a biopesticide. Efficacy and stability are two crucial factors in ensuring the effectiveness of mycopesticides [45].

The mechanisms by which biological control agents work with pathogens are diverse and intricate. They are affected by various elements of the soil environment, such as pH, humidity, temperature and the presence of other microorganisms that can antagonize the biological product. A thorough understanding of the modes of action of fungi with biocontrol properties is essential for determining how these mycopesticides should be applied. Additionally, products that have a single target of action, such as chemical pesticides, tend to have a higher risk of developing resistance, while most microbial pesticides, including mycopesticides, offer multiple mechanisms of action and are generally considered to be of low risk for the emergence of resistance [14].

Despite the complexity and versatility of their mechanisms of action, the efficacy of mycopesticides, in most situations, still does not achieve the expected results. Previous studies have highlighted the importance of the stability of these mycopesticides in relation to their efficacy [45], indicating that an unstable product results in low efficiency. Efficacy can be attributed to the instability and susceptibility of fungal propagules in environmental conditions. herefore, the selection of resistant propagules is fundamental for the creation of stable and effective mycopesticides. There are three types of propagules used as active ingredients in mycopesticides: small fragments of mycelium called hyphae, blastospores (also known as submerged conidia) and aerial conidia (from the Deuteromycota) or aerial sporidia (from the Basidiomycota) [23]. The type of propagule varies according to the production method used, which can be submerged fermentation or solid-state fermentation [45].

The successful adoption of mycopesticides depends on adequate knowledge on the part of farmers about their correct application and dosage [26,45]. Lack of education can be a barrier. Orientation, explanation and monitoring should be done regularly, proceeding to interactive questionnaire sessions that will include farmers' knowledge and learning about use, application and handling. On-farm demonstrations are the most effective method for demonstrating the efficacy of bioinputs, and it is imperative that more field data is collected because proof of effect is the key to adoption. The efforts of various government agencies to popularize the use of bioinputs can have an impact on raising the current status and application worldwide. It is important that farmers understand this form of biological pest control not simply as a substitution of conventional chemical products with biological ones. It is a more profound change that must be seen in a broader perspective, within the context of integrated management. It is therefore important that biological inputs are not marketed and used as a simple product, but rather as a technological package or control process [26].

Another important point is regulation. The regulations surrounding mycopesticides can be less clear than for traditional chemical pesticides, which can affect their approval and use. In 1994, the United State of American created the Biopesticides and Pollution Prevention Division (BPPD) to promote biopesticides as safe alternatives to chemical pesticides [26]. Biopesticides undergo an initial toxicity and ecotoxicity assessment and are more agile if there are no direct toxic effects. In 2004, Congress passed the Pesticide Registration Improvement Act (PRIA), with specific fees and deadlines. The current legislation, PRIA 4, is in effect until 2023. As of 2009, Regulation 1107/2009 replaced Council Directive 91/414, resulting in the withdrawal of more than 200 substances from the European market in a decade. This regulation opened up space for biopesticide manufacturers to fill the gap left by synthetic pesticides, especially mycopesticides, which were included in a category of low-risk products to facilitate registration [26].

In additional, to encourage the production of biopesticides, changes have been made to national and international regulations on chemical pesticides, with the aim of reducing harmful impacts on the environment and human health. Consequently, regulations are becoming stricter in terms of toxicological testing and monitoring long-term effects on human health. In Brazil, the uptake of bioinputs, such as mycopesticides, is growing. According to the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), the production of bioinputs for agriculture has grown at an average annual rate of 30% in the country, which is higher than the global average of around 18%. In an effort to encourage this scenario and facilitate adherence to the use of bioinputs in the country, government agencies have begun to look at the issue, create programs and discuss regulatory frameworks. For example, in May 2020, Decree No. 10.375 established the National Bioinputs Program (NBP), which aims to expand and strengthen the use of bioinputs for the benefit of the agricultural sector [10].

Bioinputs from micro-organisms in agriculture: environmental and economic impact

The main target for the agricultural sector nowadays is how to produce safe and enough foods with sustaining it. It could be sustained in this sector through the sustainability of different agroecosystems. The sustainability of this agroecosystem is totally controlled by the functional balance between both the productivity of plants and the processes of soils (nutrient recycling, soil microbial activity, soil organic decomposition, etc.) [39]. No doubt that soil microbes have the magic key in creating a complex network for the microbial interactions with agroecosystem or plants and soil components. This network is completely governed by several microbial and plant signals playing a great role in the communication within agroecosystems. Therefore, several interactions in agroecosystems should be studied including plant–microbe interactions, soil-microbe interactions, soil-plant interactions, plant-microbe-soil interactions [28].

Regarding different effects of bioinputs on agroecosystems, there are direct and indirect effects including increasing the crop productivity (as a direct response) and inhibition of phytopathogens by several biocontrol mechanisms like phyto-hormones synthesis, preventing plant diseases and accelerating the uptake of some soil nutrients. It is worth to mention that a strong competition between soil microbes and different strains in bioinputs could be hinted and more information regarding this competition is needed [39]. Although great efforts have been evaluated concerning these previous interrelationships and their impact on bioinputs efficacy, more investigations should be conducted on both the short- and long-term using different common methods like the analysis of soil microbial activity, soil microbial biomass, soil microbial community structure and diversity [28].

Prospects for the future of bioinputs in agriculture

Bioinputs production comprises a series of operations including mass production of the target microorganism in fermentation systems (upstream stage), followed by product formulation. In some cases, the product is directly applied after the production stage [44]. In any case, the choice of the fermentation mode is of great importance as it determines the quality and amount of the useful microbial mass and metabolites promoting plant growth and health. Biotechnology offers several well-developed fermentation schemes, which can fulfil the desired results, depending also on the formulation schemes [42]. Plant beneficial microorganisms should be easily and economically produced by the fermentation industry. The main point from an economical point of view is to ensure reasonably low-priced substrates, available locally and with stable seasonal characteristics. Despite the recent progress in the field of production and formulation of biofertilizers, additional studies are needed to further improve the upstream operational productivity using other fermentation strategies such as, a continuous mode of fermentation or different configurations of the bioreactor [43]. Here, the use of immobilized cell systems should be more actively studied as an alternative technique. Optimization of methods should be widely applied to establish highly efficient upstream processes focusing on optimized media and parameters [42]. Special attention should be paid to AMF production in fermentation systems, as they have a market potential ready to be exploited due to farmers' acknowledgement of their role in soil-plant systems development [1]. The exploitation of processes fostering the production of metabolites could also be useful to induce expression of specific metabolites necessary for the colonization of roots or enhancing the effect of the microorganisms (e.g., lipo-chitooligosaccharides) in enabling endosymbiosis [27]. However, the production of bioinputs based on non-obligate endosymbiont mycorrhizal fungi of the order Sebacinales as well as of other endophytic microorganisms would also require a specific development of fermentation and, most of all, formulation processes aiming at improving the colonization of plant tissues [1]. Optimizing the fermentation process in view of reassembling strains with differing modes of action into small communities, thereby providing more consistent protection or growth promotion than with the application of single strains, is a challenging goal worthy to venture as such strategy could widen the application of bioproducts [9].

Further development of the fermentation strategies for bioinputs production should also be focused on products able to manipulate and control phyto-microbiome structure, which will be the next biotechnological approach in the Sustainable Agriculture. However, the research aiming at optimizing the strategies for fermentation and formulation of bioinoculant must take into consideration the economic, environmental and societal features to comply with sustainability requirements [33]. In this respect, since the production cost is among the constraints expressed by farmers for the usage of bioinoculant products, possible ways to reduce it could be searched by testing new materials for the fermentation process. SSF could also exploit the use in the fermentation medium of carbon-based materials which could mostly substitute other inputs, useful to produce multifunctional inoculant [42]. Such approaches would effectively comply with sustainability conditions. Even though, the market potential of microbial-based products was confirmed by recent analyses, which valued at about 10.2 billion USD by 2025 the global biopesticide market, with an annual growth rate of approximately 15% [2] and projected 3.15 billion USD by the end of 2026 for the biofertilizers market, at an annual growth rate of about 11%, the regulatory framework could pose some bottlenecks for the sector development, including the fermentation and formulation phases [39]. Furthermore, only the drying or freeze-drying processes in the formulation of the product are allowed, which is also restrictive considering the technologies available. Nevertheless, the improvement of fermentation strategies would be useful to comply with quality and safety requirements of bioinoculant, which are the basis to increase their use in the field and thus support the transition toward a new agricultural "bio-green revolution" [17].

Conclusion

Out of the wide range of biocontrol agents, mycopesticides are interesting products because they use different modes of action to reduce plant diseases related to phytopathogenic fungi: nutrient competition, direct antagonism, mycoparasitism and resistance induction mechanisms in plants.

Despite their limitations, mycopesticides have the potential to play an important role in sustainable agriculture, especially when combined with other integrated pest management practices. As research continues to address these limitations and improve the efficacy and accessibility of mycopesticides, their adoption may increase, contributing to safer and more environmentally friendly agriculture.

Therefore, fungus-based bio-inputs are often a feasible tool to promote plant growth and ensure that plants remain healthy. However, future work should be directed towards analyzing the impact of new technologies and strategies in obtaining novel bio-inputs with a beneficial impact on agriculture and the economy.

Conflict of Interest

We declare that there are no conflicts of interest.

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