

Trichoderma harzianum-based novel formulations: potential applications for management of Next-Gen agricultural challenges

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Abstract

Fungi of the *Trichoderma* spp. genus, notably *Trichoderma harzianum*, are commonly used for biological management of deleterious seed- and soil-borne pathogens. The global biopesticides market is booming with a major share of various commercial formulations of *T. harzianum*. However, there are some major drawbacks associated with these commercial formulations including short shelf life, low on-field stability and irregular performance in different agro-climatic regions. For effectively resolving these issues, new strategies are urgently required for efficient management of pathogens. The present review provides an overview of the use of *Trichoderma* spp., with special emphasis on *T. harzianum*, and discusses future trends for biological control. Technologies are described for the microencapsulation of fungi and for the biogenic synthesis of nanoparticles, with the aim of improving the biological control of pathogens and contributing to sustainable agricultural practices.

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Keywords: biological control; eco-friendly; sustainable agriculture; nanotechnology; microencapsulation; phytopathogens; *Trichoderma harzianum*

INTRODUCTION

Biological control is considered as sustainable practice for the efficient management of notorious pathogens. Augmentation of biopesticides involves control of pathogen populations using natural enemies and act as an promising alternative to chemical pest control.^{1–7} The use of organisms in biological control has increased in popularity due to growing environmental concerns and the demand for organic produce.^{1,8–12} If the real cost of chemical pesticides were revealed, the market for biological control would increase. This is due to the fact that chemical pesticides are subsidized at the Government level and the damages (to human health and environment) caused by them are not the responsibility of the industry that produces them.¹³ According to Bourguet and Guillemaud,¹⁴ if the values of the chemical pesticides involved the real costs of production, biological control agents would have a fair competition in the market.

The *Trichoderma* genus of fungi belongs to the *Hypocreaceae* family (order: Hypocleales; class: Sordariomycetes; phylum: Ascomycota). The species of this genus are free-living soil microorganisms colonizing and decomposing litter, and also have the ability to establish symbiotic relationships with plants.^{15,16} These filamentous fungi produce a rapidly growing white mycelium that acquires a green coloration as it develops.¹⁷ Weindling¹⁸ reported for the first time that *T. lignorum* showed *in vitro* antagonistic potential against various soil-borne phytopathogens. *Trichoderma* spp. are now widely used as biological control agents

due to their effectiveness against a wide range of seed- and soil-borne phytopathogens.^{19–23} Their mechanism of action primarily includes mycoparasitism, antibiosis, competition and the induction of resistance in host plants.^{15,24–26}

Trichoderma harzianum, like other species of the genus, hosts a hydrolytic enzyme complex composed of chitinases, β -glucanases, cellulases, proteases, etc. which decompose the cell wall of phytopathogens, allowing the penetration of hyphae, colonization and the initiation of mycoparasitic activity.^{22,27} In addition, *T. harzianum* is nonpathogenic towards humans, and can be easily isolated, cultured and mass-produced at industrial scale.²⁸

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Table 1. A representative list of target organisms and crops for which *Trichoderma harzianum* and other *Trichoderma* spp. have been used as biological control agents

Biological control agents	Target organisms	Crops	References
<i>Trichoderma harzianum</i>	<i>Sclerotinia sclerotiorum</i> , <i>Phytophthora</i> spp., <i>Meloidogine javanica</i> , <i>Alternaria alternata</i> , <i>Fusarium solani</i> , <i>Macrophomina phaseolina</i>	Soybeans, potato, tomato, zucchini, eggplant, tobacco, peanut	27,107–116
<i>Trichoderma harzianum</i> T16 and T23	<i>Fusarium oxysporum</i> , <i>Fusarium solani</i> , <i>Fusarium oxysporum</i> ,	Olive Tomato	47,18,48
<i>Trichoderma harzianum</i> Ths97			
<i>Trichoderma harzianum</i> Th908			
<i>Trichoderma</i> spp.	<i>Phytium</i> spp., <i>Rhizoctonia</i> spp., <i>Bipolaris oryzae</i> , <i>Crinipallis perniciosa</i> , <i>Fusarium fujikuroi</i> , <i>Phytophthora ramorum</i>	Pea, peat moss Melon, pepper, tomato Rice Cocoa Rice	49,50,30,51–53

During interaction with phytopathogens, *T. harzianum* produces and secretes both volatile and nonvolatile secondary metabolites with antibiotic properties.²⁹ These substances act to inhibit mycelial development,³⁰ prevent the release of spores, and alter the composition of the rhizosphere to make it unsuitable for the growth and development of the phytopathogens.³¹ Furthermore, *T. harzianum* releases peptides, proteins and low molecular weight compounds¹⁶ that contribute to activation of the induced defense mechanisms of the host,^{32,33} thereby promoting its health, and enhancing root growth.³⁴

Different species and strains of *Trichoderma* spp. are used in agriculture, especially *T. harzianum*,^{35,36} and there are constant efforts to identify superior strains that possess higher antagonistic activity against phytopathogens. A representative list of the most popular strains of *Trichoderma* spp. is presented in Table 1. Several species of *Trichoderma* spp. have acquired industrial importance due to their capacity to produce large quantities of lytic enzymes and antimicrobial secondary metabolites, especially antibiotics, and various commercial products based on *Trichoderma* spp. are now marketed worldwide.^{12,37} Over 50 *Trichoderma*-based biofungicide formulations have been developed, and the genus now accounts for 60% of the fungi used as biological control agents globally, with new products constantly being registered.³⁸

Although, the biological control of phytopathogens using *T. harzianum* is a popular practice, there are certain limitations that can reduce its effectiveness, including sensitivity to ultraviolet light, and unsuitable humidity and/or temperature, which can lead to low persistence in adverse environmental conditions.^{39,40}

Following the promising results obtained on different crops, the industrial scale production of *T. harzianum* using solid and liquid substrates has increased substantially.^{38,41} Techniques used for application of live spores include foliar spraying, seed and seedling treatment, application after pruning, incorporation in

the soil and irrigation, among others. Products based on *Trichoderma* spp. are mainly aimed at controlling seed- and soil-borne phytopathogens.¹² Examples of commercial products based on *T. harzianum* and the target pathogens are shown in Table 2.

Considering the robust applications of *Trichoderma* spp. in agriculture, certain neglected aspects need to be urgently addressed, namely the maintenance of satisfactory stability during transport and storage, high viability and development of formulations with prolonged shelf life. In addition, for mass acceptance of these bioformulations, they must be cost-effective and easy to apply.⁴² In this regard, further studies are required to minimize the shortcomings of these products and to ensure their effectiveness in biological control practices.

In order to overcome the limitations of biological control techniques due to environmental factors, Singh *et al.*⁴³ suggested the development of formulations based on consortia of synergistic biological control agents that act by means of different mechanisms under both biotic and abiotic stresses. It was reported that a combination of three strains of *T. harzianum* provided successful control of an important plant pathogen affecting several crops in different climate zones.

Another promising way to enhance the activity of biological control agents is to employ novel technologies involving the use of active biomolecules such as antimicrobial proteins, lytic enzymes and bioactive secondary metabolites – that are primarily responsible for the management of phytopathogens. The development of such novel techniques to improve the results achieved using *Trichoderma*-based formulations, which have already demonstrated strong activity against important phytopathogens, is a highly promising area of research.

Micro- and nanotechnological interventions offer effective methods which reduce environmental impacts and increase agricultural productivity.^{44,45} These include techniques for the encapsulation of active ingredients of *Trichoderma* spp. in polymeric nanoparticles, for targeted and subsequent controlled release of the active biomolecules,^{46–48} nanoscale products for stimulation of the growth and development of plants^{34,49} and the synthesis of metal nanoparticles for effective management of phytopathogens.^{50–52}

Two strategies involving the use of micro- and nanotechnology to improve the performance of active ingredients are described below, together with discussion of the remaining challenges.

USE OF MICROENCAPSULATION TECHNOLOGIES TO IMPROVE THE ACTIVITY AND USE OF *TRICHODERMA* spp.

Micro- and nanotechnological interventions can be employed to improve biological control techniques by enhancing the viability of biological control agents.⁶⁸ An increasing number of recent methodologies have been studied for this purpose, including microencapsulation^{40,53,54} and nanoencapsulation.^{50,55}

Microencapsulation consists of creating a physical barrier that provides protection of the active ingredient against external factors such as mechanical stress, ultraviolet radiation, oxidation and high temperatures.^{39,56,57} As a result, the microorganism is able to survive for a longer duration and its metabolic activity is maintained, too.⁵⁸ The microencapsulation methods used may be physical (spray drying), chemical (polymerization) or physico-chemical (coacervation and ionic gelation).

Microencapsulation by spray drying is a simple, low cost and rapid technique, and the final product does not require multiple

Table 2. Representative list of commercially available products based on *Trichoderma harzianum*

Product (Brand name)	Target pathogens	Manufacturer
Tricho™	<i>Rhizoctonia</i> , <i>Sclerotinia</i> , <i>Fusarium</i> , <i>Botrytis</i> , <i>Sclerotium</i> , <i>Rosellinia</i> , <i>Pythium</i> , <i>Armillaria</i> , <i>Alternaria</i>	Orius Biotecnología (Colombia)
Natibiol™	<i>Rhizoctonia</i>	Probiagro S.A. (Venezuela)
BioFungo™	<i>Botrytis cinerea</i> , <i>Sphaerotheca pannosa</i>	Orius Biotecnología (Colombia)
ECO-77™	<i>Botrytis</i> , <i>Eutypa</i>	Plant Health Products (South Africa)
Lycomax	Soil pathogens	Russell IPM (United Kingdom)
Trichodermil™	<i>Fusarium</i> , <i>Rhizoctonia</i> , <i>Sclerotinia sclerotiorum</i> , <i>Phytophthora capsici</i> , <i>Phytophthora palmivora</i> , <i>Botrytis ricini</i>	Itaforte BioProdutos (Brazil)
Trianum™	Soil-borne pathogens	Koppert BV (The Netherlands)
ECO-T™	<i>Rhizoctonia</i> , <i>Pythium</i> , <i>Fusarium</i> , <i>Phytophthora</i>	Plant Health Products (South Africa)
Trichosav	Soil-borne pathogens	Centros de Reproducción de Medios Biológicos (Cuba)
Agroguard WG™	<i>Pythium</i> , <i>Rhizoctonia</i> , <i>Sclerotinia</i> , <i>Sclerotium</i> , <i>Phoma</i>	Life Systems Technology S.A. (Colombia)
Bioderma H	<i>Phytophthora</i> , <i>Fusarium</i> , <i>Pythium</i> , <i>Cercospora</i> , <i>Colletotrichum</i> , <i>Alternaria</i> , <i>Ascochyta</i> , <i>Macrophomina</i> , <i>Myrothecium</i> , <i>Ralstonia</i>	Biotech International Ltd. (India)
FoliGuard™	<i>Botrytis cinerea</i> , <i>Sphaerotheca pannosa</i> , <i>Oidium</i> , <i>Alternaria</i> , <i>Cladosporium</i>	Live Systems Technology S.A. (Colombia)
RootShield™/PlantShield™	<i>Pythium</i> , <i>Rhizoctonia solani</i> , <i>Fusarium</i> , <i>Sclerotinia homeocarpa</i>	Bioworks (United States)
Ecotrich ES™	<i>Rhizoctonia solani</i> , <i>Pythium</i> spp., <i>Sclerotinia</i> spp.	Ballagro Agro Tecnología Ltd. (Brazil)
Vinevax™ – Trichoprotection™	<i>Armillaria</i> , <i>Eutypa lata</i> , <i>Chondrostereum purpureum</i> , <i>Phaeomoniella chlamydospora</i> , <i>Botryosphaeria stevensii</i>	Agrimm Technologies Ltd. (New Zealand)
Antagon WP™	<i>Fusarium</i> , <i>Rhizoctonia</i> , <i>Pythium</i> , <i>Sclerotinia</i> , <i>Sclerotium</i> , <i>Botrytis</i> , <i>Ceratocystis</i> , <i>Rosellinia</i>	Bio Ecológico Ltda (Colombia)
<i>Trichoderma harzianum</i>	<i>Fusarium</i> , <i>Rhizoctonia</i> , <i>Pythium</i>	IAB S.L. (Spain)
Trichosoil	<i>Fusarium</i> spp.	Lage S.A. (Uruguay)

washing steps to eliminate residues or solvents.⁵⁹ Microparticle formation can be modified by adjusting the spray drying conditions such as the feed flow, air flow and inlet/ outlet temperatures.^{60,61} In the case of biological control agents, the spray drying method has several disadvantages. It produces microparticles of varying sizes and morphology, whereas high temperatures and rapid rates of drying can prejudice the encapsulation of conidia or viable spores.⁴⁰

The polymerization method is based on the formation of microparticles by polymerization of monomers and can be achieved by several techniques.⁶² *In situ* emulsion polymerization generally employs a medium, composed of water, monomer, surfactant and an initiation agent.⁶³ *In situ* polymerization in suspension uses a system containing one or more water-insoluble monomers and an initiator, soluble in the organic phase.⁶⁴ *In situ* interfacial polymerization involves complementary monomers and immiscible phases.⁶⁵ However, the limitations of this type of polymerization are the toxicity of unreacted monomers, the high permeability of the coating and the high fragility of the membranes.⁶⁶

The coacervation microencapsulation technique has been used to encapsulate preservatives, oils, microbial cells and enzymes.^{67–69} This method involves the phase separation of one or more polymers immiscible with the polymer coating solution, under specific pH and temperature conditions. The immiscible polymer is added to the coating polymer solution under constant stirring, leading to the formation of microparticles due to separation of the incompatible polymer followed by deposition of the coacervation phase, which surrounds the material to

be encapsulated.^{67,68} The coacervation method provides effective encapsulation and subsequent controlled release of the active agents by mechanical means or by changes in temperature or pH.⁶⁸ However, the use of this technique is limited by its high cost and the need to control the kinetic conditions.⁶⁹

Ionic gelation can be performed using extrusion, electrostatic deposition and atomization processes.⁷⁰ Normally, a polymer solution containing the active ingredient to be encapsulated is dripped into an ionic solution, under constant stirring. When the droplets enter the ionic solution, there is an instantaneous formation of spherical microparticles.^{71–73} This is a simple procedure that does not require the use of specialized equipment, organic solvents or high temperatures.^{72,74} However, a drawback of this method is the occurrence of heterogeneous microparticle gelation, due primarily to the diffusion mechanism.⁷⁵

Microencapsulation techniques can be used in various sectors, including the pharmaceutical,⁷⁶ food,⁷⁷ and cosmetics industries,⁷⁸ and in agriculture.⁷⁹

Muñoz-Celaya et al.⁸⁰ encapsulated the conidia of *T. harzianum* in polymeric carbohydrate matrices, as a way to increase the shelf life of formulations. Maltodextrin and gum arabic (in a 1:1 ratio) were used for synthesis of microparticles by the spray drying method, giving a high percentage of viable conidia (86%). The combination of these polymers resulted in a high activation energy, which together with storage at low temperatures provided stability for at least 8 weeks. Loss of viability of the conidia after this period could have been due to the oxidative stress and continued metabolic activity at 4 °C.

In other work, Jin and Custis⁷⁹ used the spray drying method for encapsulation, which prevented microbial contamination, induced dormancy and increased the shelf life of the product; however, a major disadvantage was the small number of viable encapsulated conidia. Therefore, a specific methodology was developed for the microencapsulation of *T. harzianum*, using sugars to increase the percentage of viable conidia. The inlet and outlet temperatures used in the spray drying procedure also influenced the viability of the conidia. The average sizes of the microparticles obtained were in the range of 10–25 μm and could be adjusted by varying the atomization rate. The use of 2% sucrose concentration with inlet and outlet temperatures of 60 °C and 31 °C, respectively, resulted in an improved survival rate of the conidia as compared to the free conidia.

Thus, the encapsulation of bioactive compounds through the various encapsulation methods ends up being a cost-effective method.⁸¹ The spray-drying method has ended up being the most used in the food industries^{82,83} because it is a more economical and efficient technique, has high equipment availability and low process costs. In addition, encapsulated products require fewer applications⁴⁸ when compared to the common products on the market, leading to lower costs.

USE OF SYSTEMS BASED ON *TRICHODERMA* SPP. FOR METALLIC NANOPARTICLE BIOSYNTHESIS

Due to their extremely small size, large surface area, and unique chemical, physical, and biological properties, metallic nanoparticles differ from those of the bulk materials of origin, which can provide them with the potential to control microorganisms.⁸⁴ These nanomaterials can be synthesized by chemical, physical and biogenic methods. Biogenic synthesis uses extracts of living organisms as reducing and stabilizing agents, offering the advantages of simplicity, cost-effectiveness and low environmental impact.⁸⁵

In biogenic synthesis, the reduction of precursor salts is performed using organic reducing agent molecules such as proteins, amino acids, sugars and enzymes. This process occurs naturally in the environment, because microorganisms exposed to undesired elements secrete metabolites and other compounds that convert metallic ions into elemental metal, as a form of protection.^{86,87} Fungi are widely used in this type of synthesis due to their rapid growth, ease of handling and production of large quantities of enzymes.^{88,89} However, the main disadvantage associated with the use of fungal biomolecules as reducing agents is their pathogenic behavior while using phytopathogens such as *Fusarium oxysporum*.⁹⁰ In this context, the advantage of using *Trichoderma* spp. is that they are used as biological control agent for the management of various phytopathogens. Among the biogenic nanoparticles synthesized using *Trichoderma* spp., silver nanoparticles are notable due to their known antimicrobial activity. Additionally, an advantage of biogenic synthesis is that the nanoparticles possess a stabilizing coating composed of proteins and metabolites derived from the organism, and in the case of *Trichoderma* spp., the proteins and metabolites are involved in biological control of phytopathogens, which can provide additional biological activity, stability and augment the action (Figure 1). Rodrigues *et al.*⁹¹ evaluated the filtrate of the fungi used for the synthesis of nanoparticles through SDS-PAGE and found the same protein bands for the filtrate and the nanoparticles, which indicates that the proteins from the fungi are capped around the nanoparticles.

Devi *et al.*⁹² evaluated the ability of 75 isolates of *Trichoderma* spp., including *T. harzianum*, to synthesize silver nanoparticles and obtained particles with sizes ranging from 8 to 60 nm. Singh and Raja⁹³ synthesized silver nanoparticles using filtrates of the fungi *F. oxysporum*, *Alternaria alternata*, *Phoma glomerata* and *T. harzianum*, with use of the last microorganism resulting in the most stable nanoparticles.

Ahluwalia *et al.*⁵⁵ synthesized silver nanoparticles by the reduction of silver nitrate using the filtrate of *T. harzianum* and observed the adsorption of a layer of organic compounds derived from the culture filtrate. The nanoparticles showed *in vitro* potential for the control of both Gram-positive and Gram-negative bacteria. Gherbawy *et al.*⁹⁴ also synthesized these nanoparticles and used them together with triclabendazole to control the hatching of *Fasciola* eggs in infestations of sheep and camels. It was found that the nanoparticles significantly increased the effectiveness of the pharmaceutical products.

Singh *et al.*⁹⁵ synthesized bimetallic nanoparticles composed of gold and silver using *T. harzianum* and demonstrated their catalytic properties in the reduction of methylene blue dye in the presence of NaBH_4 . Bhadwal *et al.*⁹⁶ synthesized biogenic nanoparticles of cadmium sulfide using *T. harzianum* and reported their photocatalytic potential in the degradation of methylene blue. Sundaravadivelan and Padmanabhan⁹⁷ evaluated the larvicidal and pupicidal potentials of silver nanoparticles produced using *T. harzianum* for the control of mosquito *Aedes aegypti*, and obtained promising results. Shelar and Chavan⁹⁸ synthesized silver nanoparticles using *T. harzianum* and found that it significantly increased the germination index in sunflower and soybean.

Guilger *et al.*⁵⁰ synthesized silver nanoparticles using *T. harzianum* filtrate and observed its inhibitory activity against the *in vitro* germination of sclerotia of *Sclerotinia sclerotiorum* (white mold). It was also found that the nanoparticles did not affect the growth of *T. harzianum*, enabling the fungus and the nanoparticles to be used in combination.

Nandini *et al.*⁹⁹ investigated the potential of six species of *Trichoderma* (*T. asperellum*, *T. harzianum*, *T. atroviride*, *T. virens*, *T. longibrachiatum* and *T. brevicompactum*) for the biogenic synthesis of selenium nanoparticles, and obtained positive results with all of the species. The nanoparticles varied in size and surface charges, and demonstrated potential for control of the phytopathogen *Sclerospora graminicola*, which affects pearl millet crops.

Despite the feasibility of using *Trichoderma* spp. for biogenic synthesis of nanoparticles, an important consideration is the need for regulatory structures to govern the uses of these new nanomaterials. The toxicity of nanoparticles can be significantly influenced by their properties including size, surface charge, coating and crystalline structure, as well as parameters of the environment in which they are released, such as temperature, pH, ionic strength and the amount of organic matter present.^{100,101}

Since 2004, the European Union has discussed issues related to the safety of nanomaterials and the associated ethical questions, but there is still a lack of adequate legislation. There are recognized benefits of the development of nanotechnological products, but the risks they may pose to humans and the environment must also be considered.¹⁰² Rauscher *et al.*¹⁰³ described some of the measures taken by the European Union to regulate products containing nanomaterials, including chemicals, biocides, cosmetics, foods, food packaging and medical equipment. In order to translate the potential of new nanomaterials, such as the biogenic nanoparticles synthesized using *Trichoderma* spp.,

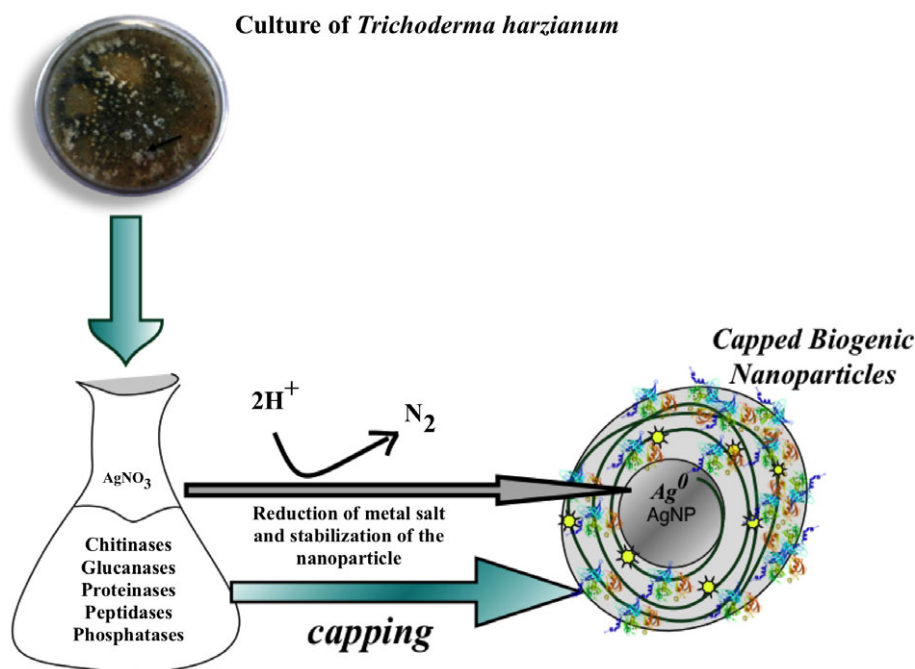


Figure 1. Schematic representation of the biogenic synthesis of silver nanoparticles using *Trichoderma harzianum*.

it will be necessary to regulate nanotechnology at an international level, establishing standardized practices for the use of nanomaterials.^{103,104}

Although nanoparticles synthesized by means of biogenic routes, such as those produced using *Trichoderma* spp., can present lower toxicity than those obtained using chemical methods because of the lack of toxic residues from the synthesis and the stabilization of the nanoparticles with organic compounds,¹⁰⁵ they can nonetheless exhibit potential cytotoxicity and genotoxicity.¹⁰⁶ However, despite the possible toxicity risks, they can offer solutions in various sectors, including in agriculture.

Given the above considerations, further exploration is needed into the potential of biogenic nanoparticles combined with *Trichoderma* spp. as biological control agents, focusing on elucidating the possible risks to human and environmental health. It is therefore vital to continue studies in this area, aiming at the future establishment of an appropriate regulatory framework.

FUTURE TRENDS, GAPS AND OBSTACLES

Fungi of the genus *Trichoderma* spp. have been widely used for the biological control of phytopathogens, although their effectiveness can be limited due to major concerns of shelf life and unreliable performance under field conditions.^{12,40} The development of new strategies, such as those described in this review, can provide ways to increase the efficacy of these fungi in biological control. The use of microencapsulation techniques can help to protect the fungi in the field and hence improve their effectiveness. Furthermore, encapsulation can extend the shelf life of the commercial products.

It is important to highlight that the development of biological products based on microencapsulation is dependent on optimization of the processes employed, whereas in some cases technological adjustments are needed in order to produce viable formulations for field applications. Techniques such as spray

drying enable the production of microparticles that present relatively homogeneous size distributions, enabling them to be more easily used in existing application systems. Another important development is the synthesis of nanoparticles using biogenic routes. Molecules and macromolecules secreted by *T. harzianum* act as a source of reducing power for the synthesis of metallic particles, whereas macromolecules such as proteins, peptides, and carbohydrates present as coatings on the particles can act as additional agents for biological control. In this context, the association of the nanotechnology with the biologically active fractions/moieties derived from *T. harzianum* on the surface of the nanoparticles can promote additional benefits for the efficient management of phytopathogens.

However, as this is a new technology, it should be stressed that detailed investigation should be conducted to confirm that these nanoparticles do not adversely affect nontarget organisms or cause environmental contamination. Moreover, to commercialize nanotechnological products obtained by the biogenic synthesis route, it is necessary to establish protocols for standardization of the preparation of these biocontrol agents, as well as methods for scaling up production processes. There is tremendous potential for the development and commercialization of novel products for the biological control of pests and pathogens based on the fungi of genus *Trichoderma* spp., especially considering their applications in sustainable agriculture.

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REFERENCES

- 1 Crowder DW and Harwood JD, Promoting biological control in a rapidly changing world. *Biol Control* **75**:1–7 (2014).
- 2 Shah PA and Pell JK, Entomopathogenic fungi as biological control agents. *Appl Microbiol Biotechnol* **61**:413–423 (2003).

- 3 de Oliveira JL, Campos EVR, Bakshi M, Abhilash PC and Fraceto LF, Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: Prospects and promises. *Biotechnol Adv* **32**:1550–1561 (2014).
- 4 Mercier J and Manker DC, Biocontrol of soil-borne diseases and plant growth enhancement in greenhouse soilless mix by the volatile-producing fungus *Muscodor albus*. *Crop Prot* **24**:355–362 (2005).
- 5 Moazami N, Biological control ☆. Reference Module in Life Sciences [Online]. Elsevier (2017). Available: <http://linkinghub.elsevier.com/retrieve/pii/B978012809633809186X> [24 October 2017]
- 6 Tylanakis JM and Binzer A, Effects of global environmental changes on parasitoid–host food webs and biological control. *Biol Control* **75**(Suppl C):77–86 (2014).
- 7 Amorim L, Rezende JAM and Filho AB, *Manual de Fitopatologia – Princípios e Conceitos*, Vol. 1. Editora Agronômica Ceres, São Paulo, p. 704 (2010).
- 8 Bettiol W, Morandi MAB, Pinto ZV, Paula Junior TJ, Corrêa EB, Moura AB *et al.*, Bioprotetores comerciais para o controle de doenças de plantas. *Rev Anual Patolog Plant* **17**:111–147 (2009).
- 9 Li Q, Ning P, Zheng L, Huang J, Li G and Hsiang T, Effects of volatile substances of *Streptomyces globosporus* JK-1 on control of *Botrytis cinerea* on tomato fruit. *Biol Control* **61**:113–120 (2012).
- 10 Tracy EF, The promise of biological control for sustainable agriculture: A stakeholder-based analysis. *J Sci Policy Agr* **5**:1–13 (2014).
- 11 Velivelli SLS, De Vos P, Kromann P, Declerck S and Prestwich BD, Biological control agents: from field to market, problems, and challenges. *Trends Biotechnol* **32**:493–496 (2014).
- 12 Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N *et al.*, *Trichoderma*-based products and their widespread use in agriculture. *Open Mycol J* **8**:71–126 (2014).
- 13 van LJC, Bolckmans K, Köhl J, Ravensberg WJ and Urbaneja A, Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl* **63**:39–59 (2018).
- 14 Bourguet D and Guillemaud T, The hidden and external costs of pesticide use [Online]. Sustainable Agriculture Reviews. Springer, Cham, pp. 35–120 (2016). Available: https://link.springer.com/chapter/10.1007/978-3-319-26777-7_2 [8 February 2018].
- 15 Howell CR, Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Dis* **87**:4–10 (2003).
- 16 Reino JL, Guerrero RF, Hernández-Galán R and Collado IG, Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochem Rev* **7**:89–123 (2007).
- 17 Domsch KH, Gams W and Anderson TH, *Compendium of Soil Fungi*, Vol. 1. Academic Press, London, p. 630 (1980).
- 18 Weindling R, *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* **22**:837–845 (1932).
- 19 Al-Hazmi AS and TariqJaveed M, Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato. *Saudi J Biol Sci* **23**:288–292 (2016).
- 20 Ben Amira M, Lopez D, Triki Mohamed A, Khouaja A, Chaar H, Fumal B, Gousset-Dupont A, Bonhomme L, Label P, Goupil P, Ribeiro S, Pujade-Renaud V, Julien JL, Auguin D, Venisse JS *et al.*, Beneficial effect of *Trichoderma harzianum* strain Ths97 in biocontrolling *Fusarium solani* causal agent of root rot disease in olive trees. *Biol Control* **110**(Suppl C):70–78 (2017), 110.
- 21 Contina JB, Dandurand LM and Knudsen GR, Use of GFP-tagged *Trichoderma harzianum* as a tool to study the biological control of the potato cyst nematode *Globodera pallida*. *Appl Soil Ecol* **115**:31–37 (2017).
- 22 Hermosa R, Viterbo A, Chet I and Monte E, Plant-beneficial effects of *Trichoderma* and its genes. *Microbiology* **158**:17–25 (2012).
- 23 Sharma V, Salwan R, Sharma PN and Kanwar SS, Elucidation of biocontrol mechanisms of *Trichoderma harzianum* against different plant fungal pathogens: Universal yet host specific response. *Int J Biol Macromol* **95**:72–79 (2017).
- 24 Bisen K, Keswani C, Mishra S, Saxena A, Rakshit A and Singh HB, Unrealized potential of seed biopriming for versatile agriculture, in *Nutrient Use Efficiency: from Basics to Advances*, ed. by Rakshit A, Singh HB and Sen A. [Online]. Springer India, New Delhi, pp. 193–206 (2015). Available: http://link.springer.com/10.1007/978-81-322-2169-2_13 [23 October 2017].
- 25 Keswani C, Bisen K, Singh SP, Sarma BK and Singh HB, A proteomic approach to understand the tripartite interactions between plant-Trichoderma-pathogen: investigating the potential for efficient biological control, in *Plant, Soil and Microbes*, ed. by Hakeem KR and Akhtar MS. [Online]. Springer International, Cham, pp. 79–93 (2016). Available: http://link.springer.com/10.1007/978-3-319-29573-2_5 [23 October 2017].
- 26 Keswani C, Mishra S, Sarma BK, Singh SP and Singh HB, Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. *Appl Microbiol Biotechnol* **98**:533–544 (2014).
- 27 Carvalho DDC, Geraldine AM, Junior ML and de Mello SCM, Controle biológico do mofo-branco por *Trichoderma harzianum* em feijão em condições de campo. *Pesqui Agropec Bras* **50**:1220–1224 (2015).
- 28 Ethur LZ, *Dinâmica populacional e ação de Trichoderma spp. no controle de fusariose em mudas de tomateiro e pepineiro*. PhD thesis, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil (2006).
- 29 Grondona I, Hermosa R, Tejada M, Gomis MD, Mateos PF, Bridge PD *et al.*, Physiological and biochemical characterization of *Trichoderma harzianum*, a biological control agent against soilborne fungal plant pathogens. *Appl Environ Microbiol* **63**:3189–3198 (1997).
- 30 Khalili E, Sadraei M, Naeimi S and Khosravi V, Biological control of rice brown spot with native isolates of three *Trichoderma* species. *Braz J Microbiol* **43**:297–305 (2012).
- 31 Benítez T, Rincón AM, Limón MC and Codón AC, Biocontrol mechanisms of *Trichoderma* strains. *Int Microbiol Off J Span Soc Microbiol* **7**:249–260 (2004).
- 32 Bisen K, Keswani C, Patel JS, Sarma BK and Singh HB, *Trichoderma* spp.: efficient inducers of systemic resistance in plants, in *Microbial-mediated Induced Systemic Resistance in Plants*, ed. by Choudhary DK and Varma A, pp. 185–195. [Online]. Springer, Singapore (2016). Available: http://link.springer.com/10.1007/978-981-10-0388-2_12 [23 October 2017].
- 33 Mastouri F, Björkman T and Harman GE, *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Mol Plant Microbe Interact* **25**:1264–1271 (2012).
- 34 Anjali CH, Sharma Y, Mukherjee A and Chandrasekaran N, Neem oil (*Azadirachta indica*) nanoemulsion-a potent larvicidal agent against *Culex quinquefasciatus*. *Pest Manage Sci* **68**:158–163 (2012).
- 35 Rifai MA, A revision of the genus *Trichoderma*, *Mycol Papers* **116**:1–56 (1969) [Online]. Available: <http://www.mycobank.org/BioMICS.aspx?TableKey=14682616000000061&Rec=2936&Fields=All> [8 February 2018].
- 36 Romão-Dumaresq AS, de Araújo WL, Talbot NJ and Thornton CR, RNA interference of endochitinases in the sugarcane endophyte *Trichoderma virens* 223 reduces its fitness as a biocontrol agent of pineapple disease. *PLOS ONE* **7**:e47888 (2012).
- 37 Błaszczyk L, Siwulski M, Sobieralski K, Lisiecka J and Jędrzycka M, *Trichoderma* spp. – application and prospects for use in organic farming and industry. *J Plant Prot Res* **54**:309–317 (2014).
- 38 Rajesh RW, Rahul MS and Ambalal NS, *Trichoderma*: A significant fungus for agriculture and environment. *Afr J Agric Res* **11**:1952–1965 (2016).
- 39 Sri S, Seethadevi A, Suria Prabha K, Muthuprasanna P and Pavitra P, Microencapsulation: A Review. *Int. J Pharma Bio Sci* **3**:509–531 (2012).
- 40 Vemmer M and Patel AV, Review of encapsulation methods suitable for microbial biological control agents. *Biol Control* **67**:380–389 (2013).
- 41 Sharma P, Sharma M, Raja M and Shanmugam V, Status of *Trichoderma* research in India: A review. *Indian Phytopathol* **67**:1–19 (2014).
- 42 Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM *et al.* From the Lab to the Farm: an industrial perspective of plant beneficial microorganisms. *Front Plant Sci* **7**:1110 (2016) [Online]. Available: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4973397/> [23 October 2017].
- 43 Singh HB, Singh BN, Singh SP and Sarma BK, Exploring different avenues of *Trichoderma* as a potent biofungicidal and plant growth promoting candidate – an overview. *Rev Plant Pathol* **5**:315–426 (2012).
- 44 Ditta A, How helpful is nanotechnology in agriculture? *Adv Nat Sci Nanosci Nanotechnol* **3**:033002 (2012).
- 45 Mishra S, Keswani C, Abhilash PC, Fraceto LF and Singh HB, Integrated Approach of Agri-nanotechnology: challenges and future trends, *Front Plant Sci* **8**:1–12 (2017) [Online]. Available: <https://www.frontiersin.org/articles/10.3389/fpls.2017.00471/full> [23 October 2017].
- 46 Grillo R, Clemente Z, Oliveira JL de, Campos EVR, Chalupe VC, Jonsson CM *et al.*, Chitosan nanoparticles loaded the herbicide

- paraquat: The influence of the aquatic humic substances on the colloidal stability and toxicity. *J Hazard Mater* **286**(Suppl C):562–572 (2015).
- 47 Grillo R, Pereira AES, Nishisaka CS, de Lima R, Oehlke K, Greiner R, Fraceto LF et al., Chitosan/tripolyphosphate nanoparticles loaded with paraquat herbicide: An environmentally safer alternative for weed control. *J Hazard Mater* **278**(Suppl C):163–171 (2014).
 - 48 Maruyama CR, Guilger M, Pascoli M, Bileshy-José N, Abhilash PC, Fraceto LF, de Lima R et al., Nanoparticles based on chitosan as carriers for the combined herbicides Imazapic and Imazapyr. *Sci Rep* **6**:srep19768 (2016).
 - 49 Giraldo JP, Landry MP, Faltermeier SM, McNicholas TP, Iverson NM, Boghossian AA et al., Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nat Mater* **13**:400–408 (2014).
 - 50 Guilger M, Pasquoto-Stigliani T, Bilesky-Jose N, Grillo R, Abhilash PC, Fraceto LF, Lima R et al., Biogenic silver nanoparticles based on *Trichoderma harzianum*: synthesis, characterization, toxicity evaluation and biological activity. *Sci Rep* **7**:srep44421 (2017).
 - 51 Mishra S, Singh BR, Singh A, Keswani C, Naqvi AH and Singh HB, Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. *PLOS ONE* **9**:e97881 (2014).
 - 52 Rao KJ and Paria S, Use of sulfur nanoparticles as a green pesticide on *Fusarium solani* and *Venturia inaequalis* phytopathogens. *RSC Adv* **10**:3 (2013).
 - 53 Ma X, Wang X, Cheng J, Nie X, Yu X, Zhao Y et al., Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficiency against *Rhizoctonia solani* in tomato. *Biol Control* **90**:34–41 (2015).
 - 54 Rathore S, Desai PM, Liew CV, Chan LW and Heng PWS, Microencapsulation of microbial cells. *J Food Eng* **116**:369–381 (2013).
 - 55 Ahluwalia V, Kumar J, Sisodia R, Shakil NA and Walia S, Green synthesis of silver nanoparticles by *Trichoderma harzianum* and their bio-efficacy evaluation against *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Ind Crops Prod* **55**:202–206 (2014).
 - 56 McLoughlin AJ, Controlled release of immobilized cells as a strategy to regulate ecological competence of inocula, in *Biotechnics/Wastewater*, pp. 1–45 [Online]. Springer, Berlin (1994). Available: <http://link.springer.com/10.1007/BFb0008732> [23 October 2017].
 - 57 Paulo F and Santos L, Design of experiments for microencapsulation applications: A review. *Mater Sci Eng C* **77**:1327–1340 (2017).
 - 58 Cassidy MB, Lee H and Trevors JT, Environmental applications of immobilized microbial cells: A review. *J Ind Microbiol* **16**:79–101 (1996).
 - 59 Morgan CA, Herman N, White PA and Vesey G, Preservation of micro-organisms by drying; A review. *J Microbiol Methods* **66**:183–193 (2006).
 - 60 O'Riordan K, Andrews D, Buckle K and Conway P, Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. *J Appl Microbiol* **91**:1059–1066 (2001).
 - 61 Vega C and Roos YH, Invited review: spray-dried dairy and dairy-like emulsions – compositional considerations. *J Dairy Sci* **89**:383–401 (2006).
 - 62 Kreuter J, *Sterilization (Microbiology): Extrusion*, Encyclopedia of Pharmaceutical Technology 20 [Online]. Scribd (1994). Available: <https://pt.scribd.com/doc/34806599/Encyclopedia-of-Pharmaceutical-Technology-Volume-20> [24 October 2017].
 - 63 Vieira RAM, Sayer C, Lima EL and Pinto JC, In-line and in situ monitoring of semi-batch emulsion copolymerizations using near-infrared spectroscopy. *J Appl Polym Sci* **84**:2670–2682 (2002).
 - 64 Machado F, Lima EL and Pinto JC, A review on suspension polymerization processes. *Polímeros* **17**:166–179 (2007).
 - 65 Costa Neto BP, Desenvolvimento de sistemas de micro e nanoestruturados de quitosana/MDI para aplicações cosméticas [Online]. Masters thesis, Universidade Federal do Rio Grande do Norte (2009). Available: <ftp://ftp.ufrn.br/pub/biblioteca/ext/bdtd/BentoPCN.pdf> [14 June 2017].
 - 66 Silva C, Ribeiro A, Ferreira D and Veiga F, Administração oral de peptídeos e proteínas: II. Aplicação de métodos de microencapsulação. *Rev Bras Ciênc Farm* **39**:1–20 (2003).
 - 67 John RP, Tyagi RD, Brar SK, Surampalli RY and Prévost D, Bio-encapsulation of microbial cells for targeted agricultural delivery. *Crit Rev Biotechnol* **31**:211–226 (2011).
 - 68 Oliveira AC, Moretti TS, Boschini C, Baliero JCC, Freitas O and Favaro-Trindade CS, Stability of microencapsulated *B. lactis* (BI 01) and *L. acidophilus* (LAC 4) by complex coacervation followed by spray drying. *J Microencapsul* **24**:685–693 (2007).
 - 69 Park JK and Chang HN, Microencapsulation of microbial cells. *Biotechnol Adv* **18**:303–319 (2000).
 - 70 Maestrelli F, Zerrouk N, Cirri M, Mennini N and Mura P, Microspheres for colonic delivery of ketoprofen-hydroxypropyl- β -cyclodextrin complex. *Eur J Pharm Sci* **34**:1–11 (2008).
 - 71 Leong J-Y, Lam W-H, Ho K-W, Voo W-P, Lee MF-X, Lim H-P et al., Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. *Particuology* **24**:44–60 (2016).
 - 72 Patil JS, Kamalapur MV, Marapur SC and Kadam DV, Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: a review. *Dig J Nanomater Biostructures* **5**:241–248 (2010).
 - 73 Souza FN, Gebara C, Ribeiro MCE, Chaves KS, Gigante ML and Grosso CRF, Production and characterization of microparticles containing pectin and whey proteins. *Food Res Int* **49**:560–566 (2012).
 - 74 Comunian TA and Favaro-Trindade CS, Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. *Food Hydrocoll* **61**:442–457 (2016).
 - 75 Burey P, Bhandari BR, Howes T and Gidley MJ, Hydrocolloid gel particles: formation, characterization, and application. *Crit Rev Food Sci Nutr* **48**:361–377 (2008).
 - 76 Agnihotri N, Mishra R, Goda C and Arora M, Microencapsulation – a novel approach in drug delivery: a review. *Indo Glob J Pharm Sci* **2**:1–20 (2012).
 - 77 Chávarri M, Marañón I, Ares R, Ibáñez FC, Marzo F and Villarán M del C, Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *Int J Food Microbiol* **142**:185–189 (2010).
 - 78 Casanova F and Santos L, Encapsulation of cosmetic active ingredients for topical application – a review. *J Microencapsul* **33**:1–17 (2016).
 - 79 Jin X and Custis D, Microencapsulating aerial conidia of *Trichoderma harzianum* through spray drying at elevated temperatures. *Biol Control. 1o de fevereiro de* **56**:202–208 (2011).
 - 80 Muñoz-Celaya AL, Ortiz-García M, Vernon-Carter EJ, Jauregui-Rincón J, Galindo E and Serrano-Carreón L, Spray-drying microencapsulation of *Trichoderma harzianum* conidia in carbohydrate polymers matrices. *Carbohydr Polym* **88**:1141–1148 (2012).
 - 81 Murugesan R and Orsat V, Spray drying for the production of nutraceutical ingredients – a review. *Food Bioprocess Technol* **5**:3–14 (2012).
 - 82 Araruna SM, Silva AH, Canuto KM, Silveira ER and Leal LKAM, Influence of process conditions on the physicochemical characteristics of cumaru (*Amburana cearensis*) powder produced by spray drying. *Rev Bras Farmacogn* **23**:132–137 (2013).
 - 83 Chang Y-X, Yang J-J, Pan R-L, Chang Q and Liao Y-H, Anti-hygroscopic effect of leucine on spray-dried herbal extract powders. *Powder Technol* **266**:388–395 (2014).
 - 84 Mody VV, Siwale R, Singh A and Mody HR, Introduction to metallic nanoparticles. *J Pharm Bioallied Sci* **2**:282–289 (2010).
 - 85 Mohanpuria P, Rana NK and Yadav SK, Biosynthesis of nanoparticles: technological concepts and future applications. *J Nanoparticle Res* **10**:507–517 (2008).
 - 86 Lloyd JR, Microbial reduction of metals and radionuclides. *FEMS Microbiol Rev* **27**:411–425 (2003).
 - 87 Siddiqi KS and Husen A, Fabrication of metal nanoparticles from fungi and metal salts: scope and application, *Nanoscale Res Lett* **11**:1–15 (2016) [Online]. Available: <http://www.nanoscalereslett.com/content/11/1/98> [23 October 2017].
 - 88 Alghuthaymi MA, Almoammar H, Rai M, Said-Galiev E and Abd-Elsalam KA, Myconanoparticles: synthesis and their role in phytopathogens management. *Biotechnol Biotechnol Equip* **29**:221–236 (2015).
 - 89 Dhillon GS, Brar SK, Kaur S and Verma M, Green approach for nanoparticle biosynthesis by fungi: current trends and applications. *Crit Rev Biotechnol* **32**:49–73 (2012).
 - 90 Pantidos N and Horsfall LE, Biological synthesis of metallic nanoparticles by bacteria, fungi and plants, *J Nanomedicine Nanotechnol* **5**:1–10 (2000) [Online]. Available: <https://www.omicsonline.org/>

- open-access/biological-synthesis-of-metallic-nanoparticles-by-bacteria-fungi-and-plants-2157-7439.1000233.php?aid=31363 [23 October 2017].
- 91 Rodrigues AG, Ping LY, Marcato PD, Alves OL, Silva MCP, Ruiz RC *et al.*, Biogenic antimicrobial silver nanoparticles produced by fungi. *Appl Microbiol Biotechnol* **97**:775–782 (2013).
 - 92 Devi TP, Kulanthaivel S, Kamil D, Borah JL, Prabhakaran N and Srinivasa N, Biosynthesis of silver nanoparticles from *Trichoderma* species. *Indian J Exp Biol* **51**:543–547 (2013).
 - 93 Singh P and Raja RB, Biological synthesis and characterization of silver nanoparticles using the fungus *Trichoderma harzianum*. *Asian J Exp Biol Sci* **2**:600–605 (2011).
 - 94 Gherbawy YA, Shalaby IM, Abd El-sadek MS, Elhariry HM and Banaja AA, The anti-fasciolosis properties of silver nanoparticles produced by *Trichoderma harzianum* and their improvement of the anti-fasciolosis drug Triclabendazole. *Int J Mol Sci* **14**:21 887–21 898 (2013).
 - 95 Singh P, Gupta RK, Shrivastav A, Bhadwal AS, Shrivastav BR and Tripathi RM, Fungal biomolecules assisted biosynthesis of Au–Ag alloy nanoparticles and evaluation of their catalytic property. *IET Nanobiotechnol* **9**:178–183 (2015).
 - 96 Bhadwal AS, Tripathi RM, Gupta RK, Kumar N, Singh RP and Shrivastav A, Biogenic synthesis and photocatalytic activity of CdS nanoparticles. *RSC Adv* **4**:9484–9490 (2014).
 - 97 Sundaravadivelan C and Padmanabhan MN, Effect of mycosynthesized silver nanoparticles from filtrate of *Trichoderma harzianum* against larvae and pupa of dengue vector *Aedes aegypti* L. *Environ Sci Pollut Res* **21**:4624–4633 (2014).
 - 98 Shelar GB and Chavan AM, Myco-synthesis of silver nanoparticles from *Trichoderma harzianum* and its impact on germination status of oil seed. *Biollife* **3**:109–113 (2015).
 - 99 Nandini B, Hariprasad P, Prakash HS, Shetty HS and Geetha N, Trichogenic-selenium nanoparticles enhance disease suppressive ability of *Trichoderma* against downy mildew disease caused by *Sclerospora graminicola* in pearl millet. *Sci Rep* **7**:2612 (2017) [Online]. Available: <http://www.nature.com/articles/s41598-017-02737-6> [23 October 2017].
 - 100 Walters C, Pool E and Somers V, Nanotoxicology: A Review, in *Toxicology - New Aspects to This Scientific Conundrum*, ed. by Soloneski S and Larramendy M. [Online]. InTech, Croatia (2016). Available: <http://www.intechopen.com/books/toxicology-new-aspects-to-this-scientific-conundrum/nanotoxicology-a-review> [23 October 2017].
 - 101 McGillicuddy E, Murray I, Kavanagh S, Morrison L, Fogarty A, Cormican M *et al.*, Silver nanoparticles in the environment: Sources, detection and ecotoxicology. *Sci Total Environ* **575**:231–246 (2017).
 - 102 Baran A, Nanotechnology: legal and ethical issues. *Ekon Zarzadzanie* **8** (2016) [Online]. Available: <https://www.degruyter.com/view/j/emj.2016.8.issue-1/emj-2016-0005/emj-2016-0005.xml> [23 October 2017].
 - 103 Rauscher H, Rasmussen K and Sokull-Klüttgen B, Regulatory Aspects of Nanomaterials in the EU. *Chem Ing Tech* **89**:224–231 (2017).
 - 104 Baran A, Prawne aspekty nanotechnologii w kontekście ochrony środowiska, *Ekon Śr* **1**:27–40 (2015) [Online]. Available: <http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.baztech-49b8c275-1160-46dd-8bf6-0856a0aaa40f> [23 October 2017].
 - 105 Sharma D, Kanchi S and Bisetty K, Biogenic synthesis of nanoparticles: A review. *Arab J Chem* (2015) [Online]. Available: <http://www.sciencedirect.com/science/article/pii/S1878535215003147> [8 February 2018].
 - 106 de Lima R, Seabra AB and Durán N, Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles. *J Appl Toxicol* **32**:867–879 (2012).
 - 107 Abdullah MT, Ali NY and Suleman P, Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. *Crop Prot* **27**:1354–1359 (2008).
 - 108 Bae S-J, Mohanta TK, Chung JY, Ryu M, Park G, Shim S *et al.*, *Trichoderma* metabolites as biological control agents against *Phytophthora* pathogens. *Biol Control* **92**:128–138 (2016).
 - 109 Etebarian HR, Scott ES and Wicks TJ, *Trichoderma harzianum* T39 and *T. virens* DAR 74290 as potential biological control agents for *Phytophthora erythroseptica*. *Eur J Plant Pathol* **106**:329–337 (2000).
 - 110 Ezziyyani M, Requena ME, Egea-Gilabert C and Candela ME, Biological control of *Phytophthora* root rot of pepper using *Trichoderma harzianum* and *Streptomyces rochei* in combination. *J Phytopathol* **155**:342–349 (2007).
 - 111 Gveroska B and Ziberoski J, *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternata* on tobacco. *Appl Technol Innov* **7**:67–76 (2012).
 - 112 Khaleli N and Taheri P, Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. *J Plant Prot Res* **56**:21–31 (2016).
 - 113 Rojo FG, Reynoso MM, Ferez M, Chulze SN and Torres AM, Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Prot* **26**:549–555 (2007).
 - 114 Sharon E, Bar-Eyal M, Chet I, Herrera-Estrella A, Kleifeld O and Spiegel Y, Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology* **91**:687–693 (2001).
 - 115 Zancan WLA, Machado J da C, de Sousa BFM and de Matos C de SM, Crescimento micelial, produção e germinação de escleródios de *Sclerotinia sclerotiorum* na presença de fungicidas químicos e *Trichoderma harzianum*. *Biosci J* **28**:782–789 (2012) [Internet]. Available: <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/13909> [23 October 2017].
 - 116 Zhang F, Ge H, Zhang F, Guo N, Wang Y, Chen L *et al.*, Biocontrol potential of *Trichoderma harzianum* isolate T-aloe against *Sclerotinia sclerotiorum* in soybean. *Plant Physiol Biochem* **100**:64–74 (2016).