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The Biosynthesis of Nanoparticles by Fungi and the Role of Nanoparticles in Resisting of Pathogenic Fungi to Plants: A Review

Eman K. Abdul-Karim* & Halima Z. Hussein**
Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Iraq
*** Principal Correspondence, author email:** eman.khalil@coagri.uobaghdad.edu.iq
**** Corresponding author email:** drhalima@coagri.uobaghdad.edu.iq

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Abstract: This study aimed to demonstrate the activity of nanomaterials, the mechanisms of their biosynthesis, methods of measurement, and the factors that roles their biosynthesis by fungi. Moreover, focusing on their impact on host resistance against fungal pathogens. Nanometerials have been considered as one of scientific research priorities due to their new features (melting temperature, binding energy, electronic structure and catalytic activity, magnetic properties, dissolving temperature, and hardness). The performance and efficiency of nanomaterials compared to their normal state has been proven in many fields such as health care, agriculture, transportation, energy, information and communication technology. Many mechanical, chemical and physical methods were implemented to produce nanoparticles, which are considered as unsafe, expensive and environmentally dangerous. Therefore, researchers interested in biosynthesis of nanoparticles using fungi, bacteria or plants systems to make the process environmentally and economically safe. Furthermore, microorganisms such as yeasts, fungi and bacteria efficiency of converting inorganic ions into metallic nanomaterials was well studied. In agriculture, studies have confirmed impact of nanoparticles in improving plant productivity and pathogens resistance in different approaches like direct spraying on plants, soil, and stored fruits in a curative and preventive modes.

Keywords: Biosynthesis, nanoparticles, Fungi

Introduction

Nanotechnology is an advanced field that relies on the synthesis of nanoparticles or ultrafine particles. The word nano is derived from the Greek word for dwarf, and is equal to one billionth of matter (Sparks *et al*. 2015). Narayanan & Sakthivel (2010) reported that nanotechnology usually deals with size of particles between 1-100 nanometers (nm), while the scientists previously have been dealing with micrometric-sized particles in many fields, and recently nanotechnology replaces micro technology. Nanotechnology effectivity has proven the in many fields including medicine, engineering, science, agriculture and electronics

(Ahmed *et al*., 2017; Al-Musawi & Al-Saadi, 2021). Interestingly, the life cells contain many natural nanoparticles such as enzymes, ribosomes, and Golgi bodies (Dutta, 2012).

The potential of nanoparticles depends on the distinct biological, chemical, physical and electrical properties of these materials, as well as the structural rigidity of the nanoparticles and despite of their small atomic structure, their activity increases over their normal size by rearranging the atoms structure, to make new particles with new features (Sassolas *et al*., 2011; Bakshi *et al*., 2014). The new features indeed, return to the surface area, as the number of atoms present on the surface of the particle increases with the increase in the surface area, which is responsible for the chemical interaction between the nano-molecule and other molecules due free electrons that are not bound inside the particle, the new status can explain the change in the properties of the nanoparticle as well as quantum effect that roles the molecules in their new state.

Notably, the new characteristics of nanoparticles allow it to influence through its shape and size, and direct it to interact with the target tissues (Gul *et al*., 2014). Additionally, Tawfeeq (2014) introduced the term small nanoparticles to refer to the particles with the size ranges between 1-100 nm, while the particles with sizes of more than 100 nm are called large nanoparticles. Depending on the dimensions of nanoparticles, they are classified into three types: one-dimensional nanoparticles that represent all particles that one of its dimensions is less than 100 nm, such as thin films that used in the food industry; the second type is two-dimensional nanoparticles that have two dimensions ranging from 1-100 nm such as nano-fibres, whereas the third type that have three dimensions with a size of less than 100 nm are called three-dimensional nanoparticles such as fluorine. Furthermore, nanoparticles are divided into two major groups, the first one includes organic nanoparticles such as carbon nanoparticles and the second group is inorganic nanoparticles such as noble metal nanoparticles (Xu *et al*., 2006).

Techniques of the nanoparticles production

Nanoparticles are produced by two main techniques, the first method is by reducing large-sized materials to very tiny parts (within the nanoscale), and this is called the top to the bottom technique by grinding, scraping, or using laser. While the second method includes the collecting atoms and molecules that have been separated from each other, and then grouped to reach the nanoscale size in a composition that is subject to the nature of the particulate matter, and this method is called from the bottom to top by sol-gel or aerosol. Furthermore, there are several methods to convert the materials into nanoscale sizes, including chemical methods that are characterized by their high cost, toxicity, the purification problems, and time consumption (Tran *et al*., 2013); whereas the physical method, which is depending on evaporative condensation, consumes a high energy rate and wasting time, while the Laser ablation method produce pure colloidal materials and not needs a chemical agents (Tsuji *et al*., 2002).

Interestingly, using of chemical and physical methods requires reducing agents, inhibitors, and protective agents, which are often toxic and flammable in addition to their low yield (Bar *et al.* 2009 a,b, Sharma *et al*. 2009;), and also the produced particles often larger than the sizes of biologically synthesized particles (Joerger *et al*., 2000). The biological method or the biosynthesis of the nanoparticles that depends on micro-organisms (fungi, bacteria, viruses, and algae) and plants (Prasad *et al*., 2018; Alhilfi *et al*., 2021) where their active substances such as enzymes, proteins, amino acids, sugars and vitamins act as reducing agents, anti-clumping and stabilizing nanoparticles to produce inorganic nanoparticles such as gold, silver, calcium, silicon, iron oxides, zinc and titanium due to their distinctive properties as well as the ability to produce nanoparticles outside and inside the cell (Asmathunisha & Kathiresan, 2013) under a certain conditions including pressure, temperature, the concentration of ions and pH; thus it is characterized by low cost, easiness, energy saving and environmentally safe method (Kathiresan *et al*., 2010). Moreover, several microorganisms have demonstrated their efficiency in absorbing and accumulating inorganic metal ions from their environment, and also the ability to use their original biochemical processes to convert ions of inorganic particles into metallic nanoparticles (Baker *et al*., 2013).

The biological synthesis of nanoparticles

Nanoparticles are synthesized by consuming microorganisms the ions that to be converted into nanoscale sizes, by converting them into metals in the presence of enzymes resulting from cell activities due to the electron transfer. Additionally, the internal or external formation of nanoparticles is actually depending on the location in which they are formed (Mann, 2001), as the formation of nanoparticles occurs in the presence of enzymes inside the cells as a result of the electrostatic interaction between metal ions and the positively charged groups in the enzymes (proteins) of the cell wall (Kashyap *et al*., 2013). The metal ions are trapped on the surface of cells and reduce ions in the presence of enzymes if they are formed outside cells (Zhang *et al*., 2011). In constant, Rai *et al*. (2011) reported some hypotheses explaining the mechanism for biosynthesis innate nanoparticles, as it includes three steps: metal ion trapping, biological reduction, and then particle synthesis, where the reduction of metal ions to metal atoms occurs; the reduction occurs through reduction enzymes such as Nitrate reductase, which depends on Quinctase enzyme or NADPH or both.

The reduction process involve several stages with the assistance of cell wall enzymes, as the negative ions are adsorbed on the surface of the fungal cells, and then the reduction of the metal ions into the neutral atomic form are performed, after that the nanoparticles are formed and aggregate on the walls of the fungal cells, however in some cases the ions can pass inside the fungal cells and thus are reduced by the action of enzymes on the cell membrane or within the cytoplasm, for example the silver ion acts as a substrate binds to the reduction enzyme, as it converts NADPH into NADP to release electrons that implemented in the conversion of the material into the nano state that depends on the proteins produced by fungi for the biological reduction of metal ions into nanoparticles (Jain *et al*., 2010).

Chen *et al*. (2003) demonstrated that the adsorption of *Phoma* sp. hyphae with 13 mg of silver for 50 hours, produced silver nanoparticles with 70 nm in size. Furthermore, the SH-containing proteins produced from *Coriolus versicolor* were involved in the production of cadmium nanoparticles (Sanghi &

Verma, 2009). Vahabi *et al*. (2011) also produced silver nanoparticles of 5-50 nm size by mixing 1 Mm of silver nitrate with 10g of *Trichoderma reesei* biomass with shaking at 100 rpm for 120 hours at 28°C. In addition, silver nanoparticles were produced by treating 1 Mm of silver nitrate with *Aspergillus foetidus* extract and incubated at 28±2° C with shaking at 150 rpm in the dark; the produced silver nanoparticles size was in the range of 20-40 nm (Roy *et al*., 2013).

Ramalingmam *et al*. (2015) revealed that *Curvularia lunata* can used in silver nanoparticles biosynthesis using silver nitrate solution, and the aqueous silver (Ag+) ions also produced stable AgNPs after exposing to a filtrate of *C. lunata*. Rajan *et al*. (2016) also produced spherical nanoparticles by adding 1 Mm of zinc nitrate salt to filtrate suspension of *Aspergillus fumigatus*, and adjusting pH to 6.5 and incubating at 32°C with shaking at 150 rpm for 72 hours.

Nanoparticles measurements

Many instruments usually used to examine the nanoparticles properties. The Fourier-transform infrared spectroscopy (FTIR) used to determine

the types of chemical bonds and to analyze the functional groups of the nanoparticles; while the size of the nanoparticles, the crystal structure and surface appearance are determine using XRD, transmission electron microscope (TEM) and the electron microscope. The SEM scanner uses the Energy-Dispersive X-ray Spectroscopy (EDX) for the initial analysis of the chemical characterization of the nanoparticles, while the Atomic Force Microscopy (AFM) and Scanning force microscope (SFM) are used for imaging of the atoms and structures, the Dynamic light scattering (DLS) analysis is used to determine the size distribution of the nanoparticles.

Table (1): Some types of fungi used in the production of nanoparticle.

No.	Fungi	The Nanoparticle	Reference
1	*Aspergillus niger*	Ag	Gade *et al*. (2008)
2	*Fusarium oxysporum*	Bi_2O_3	Uddin *et al*. (2008)
3	*Penicillium fellutanum*	Ag	Kathiresan *et al*. (2009)
4	*Alternaria alternata*	Se	Sarkar *et al*. (2011)
5	*Aspergillus flavus*	TiO_2	Rajakumar *et al*. (2012)
6	*Lentinus edodes*	Au	Vetchinkina *et al.* (2014)
7	*Penicillium expansum*	Ag	Mohammadi & Salouti (2015)
8	*Curvularia lunata*	Ag	Ramalingmam *et al*. (2015)
9	*Trichoderma viride*	Ag	Elgorban *et al*. (2016)
10	*Rhizopus stolonifer*	Ag	Abdel Rahim *et al*. (2017)

The fungal biosynthesis of nanoparticles

Several studies have been conducted to produce nanoparticles by fungi, as Yadav *et al*. (2015) demonstrated the ability of fungi to produce the nanoparticles (Table 1).

The fungi mostly characterized by their ability to produce large quantities of enzymes and proteins that contribute in the production of nanoparticles (Mohanpuria *et al*., 2008), as they produce them in high levels that stimulate the production of nanoparticles from minerals rapidly (Rai *et al*., 2009) as well as a rapid growth of fungi and ease of handling in the laboratory (Castro-Longoria *et al*., 2011) that are depends on protein, organic acids, hydrogenase, and nitrate-dependent reduction (Bakhi *et al*., 2017). Furthermore, Jha & Prasad (2016) reported that *Aspergillus* and *Penicillium* species possess many hydroxy and methoxy derivatives from benoquinone and toluquinone as a result of exposure to metal stress that undergoes oxidation and reduction reactions to produce nanoparticles (Jha & Prasad, 2010). Furthermore, fungi possessing a specific gene control the secretion of high quantities of enzymes needed for nano converting, which can obtain many numbers of nanoparticles in small sizes (Jha & Prasad, 2016). Additionally, accumulated studies observed that many fungi can produce nanoparticles such as *Aspergillus* sp., *Cladosporum* sp., *Penicillium* sp., *Fusarium* sp. and *Trichothecium* sp. (Bakhi *et al*., 2017).

The fungi can reduce the size of metal ions to nanoparticles inside the fungal cell through the interaction of the fungus biomass with the metal, and outside the fungal cell from the interaction of the fungus filtrate with the mineral solution (Yadav *et al*., 2015).

This can performed via two different mechanisms; the first one through the fungal cell wall by trapping of metal ions on the surface of the fungal cell due to the electrostatic interaction of the positively charged groups in the enzymes presented in the fungal cell wall, after that the enzymes inside the cell are reduce the metal ions that work to accumulate metal ions and form nanoparticles, and their presence was noticed on the cytoplasmic membrane and the cytoplasm, and the small particles spread across the fungal cell wall. Whereas the second mechanism includes the reduction of nitrates depending on NADPH secreted by fungi in the reaction medium to produce extracellular nanoparticles by converting it to NADP (Fig. 1).

Ahmad *et al*. (2002) determined the ability of *Fusarium oxysporum* to reduce sulfites enzyme to produce cadmium sulfide nanoparticles. In addition, Ahmed *et al*. (2003) also showed the possibility of producing silver nanoparticles by *F. oxysporum* with a size of 5-50 nm. Gold nanoparticles also were produced in various spherical, triangular and hexagonal shapes with a size of 8-40 nm using *Colletotrichum* sp.; it was found that the fungus proteins have an important role in stabilizing the gold nanoparticles (Shankar *et al*., 2003).

Duran *et al*. (2005) observed that *F. oxysporum* had the ability to produce the spherical silver nanoparticles within size ranged between 20-50 nm in diameter. Furthermore, Bhainsa & D'Souza (2006) mentioned the possibility of silver nanoparticles biosynthesis in size 5-25 nm in the presence of *Aspergillus fumigatus*.

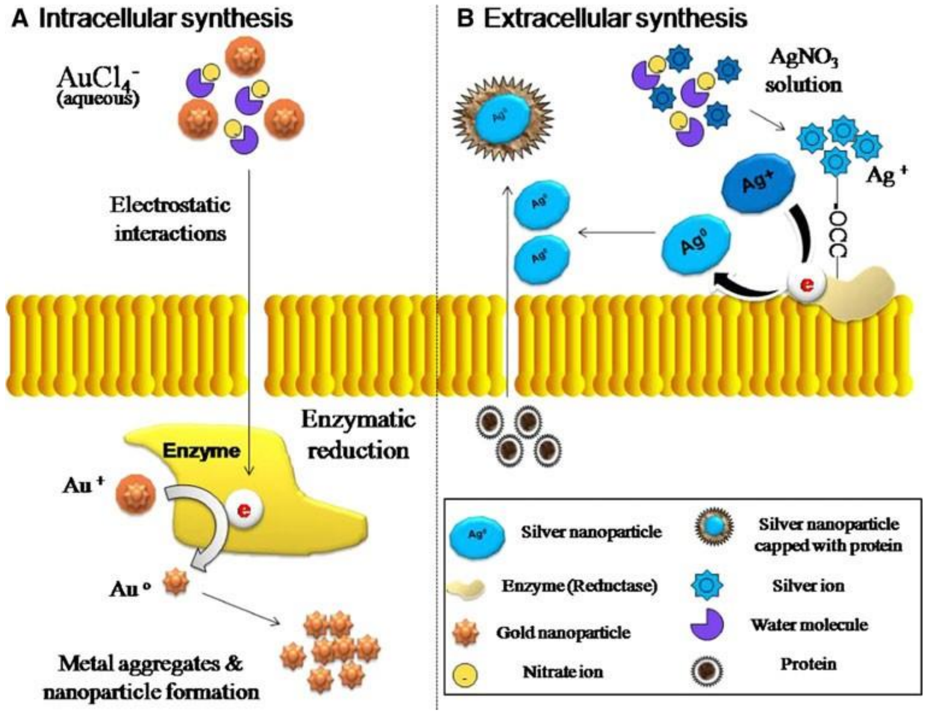

Fig (1): Mechanism of intracellular and extracellular synthesis of gold (Au) and silver (Ag) Nanoparticles through fungi (Kashyap *et al*., 2013)

Additionally, Gericke & Pinches (2006 b) reported the production spherical, triangular, hexagonal and other shapes of gold nanoparticles using *Verticillium luteoalbum*. Whereas Mukherjee *et al*. (2008) pointed out the possibility of producing silver nanoparticles using *Trichoderma asperellum* filtrate after exposing to silver nitrate. Gade *et al*. (2008) indicated that *Aspergillus niger* has the ability to produce silver nanoparticles.

The silver nanoparticles were also produced using the *Cladosporium cladosporioides* (Balaji *et al*. 2009), while Varshney *et al*. (2009) demonstrated that *Fusarium semitectu* was sufficiently produced silver nanoparticles at a size of 10-60 nm. Kathiresan *et al*. (2009) also showed that the spherical silver nanoparticles were produced using *Penicillium fellutanum* filtrate; whereas Varshney *et al*. (2009) observed the production of silver nanoparticles in different shapes from triangle to spherical with a size ranged between 20-80 nm by *Hormoconis resinae*. Moreover, the silver nanoparticles at size of 10-25 nm were produced using *Rhizopus stolonifer* (Binupriya *et al*., 2010).

Ray *et al*. (2011) reported that the use of *Tricholoma crissum* to produce the silver nanoparticles resulted in the formation of spherical particles with a small number of hexagonal particles. Selenium nanoparticles also were formed in size of 15-30nm by *Alternaria alternata* filtrate (Sarkar *et al*., 2011). Furthermore, *Chrysoporium tropicum* was used to synthesize gold and silver nanoparticles at a size of 2-15 and 20-50 nm respectively (Soni & Prakash, 2012). Rajakumar *et al*. (2012) also mentioned the using of *Aspergillus flavus* to produce titanium nanoparticles. In contrast, gold nanoparticles were synthesized using *Penicillium chrysogenum* and *Rhizopus oryza* (Sheikhloo &

Salouti, 2011; Sheikhloo *et al*., 2012). The zinc nanoparticles were produced using *Saccharomyces cerevisiae* MTCC2918 with a size of 30-40nm (Mala & Rose, 2014).

Factors affecting the production of nanoparticles by fungi

Various factors including temperature, biomass, concentration, exposure time, pH, and presence of enzymes are affect the shape and size of produced nanoparticles (Kashyap *et al*., 2013). Armendariz *et al*. (2004) revealed that pH is an effective factor in the nature and size of nanoparticles produced using fungi. Fayaz *et al*. (2009) showed that increasing the reaction temperature leads to a decrease in the size of the synthesized nanoparticles. Dhillon *et al*. (2012) evaluated the importance of temperature in the regulation of fungus activity and ion movement during the production of nanoparticles. Additionally, Darroudi *et al*. (2011) found that the time period greatly influences on the production and quality of nanoparticles; Khan *et al*. (2016) studied the impact of pH, amount of fungal biomass, temperature, and silver nitrate concentration on the production of silver nanoparticles using *Aspergillus niger* and observed that the improvement of these factors can improve the production of silver nanoparticles.

Application of nanomaterials in plant pathology

1. fungal growth inhibitor

The impact of nanoparticles was covered widely by previous studies. Oh *et al*. (2006) demonstrated the activity of silver nanoparticles in inhibiting *Botrytis cinerea* growth. Furthermore, silver nanoparticles have been shown a notable inhibition activity to *Phoma glomerata*, *Phoma herbarum*, and *Fusarium semitecum* (Gajbhiye *et al*., 2009). The silver nanoparticles also inhibited the stony bodies' growth of *Fusarium* spp. (Min *et al*., 2009). In contrast, Aguilar-Mendez *et al*. (2011) determined the efficacy of silver nanoparticles as inhibitors of the mycelial growth of *Colletotricum gloespoides*, as well as inhibiting the growth of *Pencillium phoenicum*, *Aspergillus niger*, and *Aureobasidium pullulans* (Khaydarov *et al*., 2012).

Saharan *et al*. (2013) found that chitosan nanoparticles effectively inhibited the mycelial growth of *Alternaria alternata*, *Macrophomina phaseolina*, and *Rhizoctonia solani*.

The chitosan nanoparticles showed high activity of inhibiting to the growth of *F. oxysporum*, *Alternaria terrens* and *Fusarium solani* (Sahab *et al*., 2015). El-Argawy *et al*. (2017) found that magnesium oxide nanoparticles inhibited the growth of *F. oxysporum*, *Sclerotium rolfsii*, and *R. solani in vitro*. Additionally, Ahmed (2017) revealed that chitosan nanoparticles moderately inhibited the growth of *Botrytis fabae* and *Alternaria alternata* under laboratory conditions. The use of chitosan nanoparticles achieved inhibition of the *Verticilium dahlia* growth, as well as it decreased the spore germination percentage (Xing *et al*., 2017). In addition, nano-chitosan was actively inhibited the growth of *Neoscytalidium dimidiatum in vitro* when mixed with nano-silver compared with using the silver alone (Ngoc *et al*., 2018).

Abdul-Karim (2020) reported the activity of magnesium oxide and chitosan nanoparticles growth inhibitors to *Neoscytalidium hyalinum*, *N. novaehollandiae* and *N. dimidiatum*. Al-Tamimi *et al*. (2020) observed a high activity of chitosan nanoparticles to inhibit the growth of *Alternaria solani* compared to chitosan treatment alone.

Moreover, the chitosan nanoparticles activity was elevated with the increase of the concentration. Recently, Hussain & Hussein (2020) clarified that using of the crude extracts nanoparticles of *Agaricus* and *Pleurotus* inhibited the growth of *A. flavus*, comparing with the normal extracts. Additionally, the treatment of contaminated corn seeds with nano-extracts significantly reduced aflatoxin production. The results revealed the effectiveness of nano-magnesium oxide in inhibiting the growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro , as the inhibition percentages were 98.07, 98.43 and 100% at concentrations of 1, 2 and 3g.100ml^{-1} (Abdul-Karim, 2021).

2. Control of pathogenic fungi

Suryadi *et al*. (2017) demonstrated the role of chitosan nanoparticles in inhibiting the germination of *Colletotrichum gloeosporioides* and reducing the anthracnose on *Carica papaya* plant by when used as protective agent. Moreover, the using magnesium oxide nanoparticles reduced the severity of root rot disease caused by *Fusarium oxysporum*, *Sclerotium rolfsii* and *R. solani* (El-Argawy *et al*., 2017).

Ahmed (2017) showed that the chitosan nanoparticles was effective in reducing the disease severity of *Botrytis fabae* and *A. alternata* in broad bean (*Vicia faba*). On the other hand, Hussen & Hussein (2016) observed that the using of magnesium oxide nanoparticles at a low concentration was effective in reducing the incidence and severity of infection with *F. solani* f. sp. *cucurbitae* on watermelon; also it was effective in reducing the severity of *Alternaria alternata* and *Botrytis fabae* infection on the broad bean (Ahmed, 2017). Interestingly, Ali *et al*. (2018) observed that magnesium oxide nanoparticles reduced the severity of root rot and seedling disease caused by *R. solani* on eggplant plants (*Solanum melongena*). Abdul-Karim (2020) reported that the impact of magnesium oxide and chitosan nanoparticles in reducing the severity of the stem black rot and wilting of the branches infection. Al-Tamimi *et al*. (2020) observed that chitosan nanoparticles reduced the severity of *A. solani* infection, furthermore the plant growth was increased significantly with notable stimulation of the plant systemic resistance.

Conclusion

This article shows the importance of manufacturing nanoparticles by fungi due to its environmentally friendly, low cost and high efficiency in converting materials into nanoscale sizes compared to other methods, as well as its high efficiency in resisting plant pathogens and inducing systemic resistance in plants.

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ORCID

E. K. Abdul-Karim https://orcid.org/0000-0003-0353-8757

H. Z. Hussein https://orcid.org/0000-0003-3068-9999\

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التخليق الحيوي للجسيمات النانوية بواسطة الفطريات ودورالجسيمات النانوية في مقاومة الفطريات المسببة لامراض النباتات : مراجعة بحثية

ايمان خليل عبد الكريم وحليمة زغير حسين

قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، العراق

المستخلص: هدفت هذه الدراسة الى توضيح فعالية المواد النانوية والية تصنيعها بايولوجياً وطرق قياسها والعوامل التي تحدد طريقة تصنيعها بواسطة الفطريات ،وذكر بعض الدراسات التي اوضحت فعاليتها في مقاومة المسببات المرضية الفطرية، اذ اصبحت الجسيمات النانوية من اولويات البحث العلمي لما تتمتع به من خواص جديدة (حرارة الانصهار، طاقة الربط ، التركيب الالكتروني ونشاط التحفيز ، الخصائص المغناطيسية ، درجة حرارة الذوبان ، قوة الصلابة وغيرها الكثير) ينعكس تأثيرها على فعاليتها وكفاءتها مقارنة بالمواد بحجمها الطبيعي، واثبتت فعاليتها في الكثير من المجالات مثل الرعاية الصحية والزراعة والنقل والطاقة وتكنولوجيا المعلومات والاتصالات . تستخدم طرائق عديدة لانتاج الجسيمات النانوية ميكانيكيا وكيميائيا وفيزيائيا ولكنها تعد غيرآمنه على البيئة ومكلفة لذا اتجه الباحثون الى إيجاد طرائق آمنه لانتاج الجسيمات النانوية مثل استعمال الفطريات أو البكتريا أو النباتات اذ تكون الجسيمات المصنعه بها امنه بيئياً واقتصادياً وأثبتت الكائنات الحية الدقيقة مثل الخمائر والفطريات والبكتريا كفاءتها على استعمال عملياتها البيوكيميائية الاصلية لتحويل ايونات المواد غير العضوية الى مواد نانوية معدنية. اما في مجال الزراعة اثبتت الدراسات فعاليتها في تحسين انتاجية النباتات ومقاومة المسببات المرضية التي تصيب النبات وبأكثرمن طريقة كأستخدامها بطريقة الرش مباشرة على النبات او معاملة البذور قبل الزراعة اواستخدامها مع التربة او معاملة الثمار للحفاظ عليها اثناء التخزين فضلاً عن فعاليتها بطريقة علاجية ووقائية ضد المسببات المرضية التي تصيب النباتات.

الكلمات المفتاحية: التخليق الحيوي، الجسيمات النانوية، الفطريات.