



# IJEAST

INTERNATIONAL JOURNAL  
OF ENGINEERING APPLIED SCIENCE  
AND TECHNOLOGY



**VOLUME : 1      ISSUE : 12      Print / Issue Publication Date: 30-Nov-2016**



**ISSN : 2455-2143**



Indexed In



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# BIOSYNTHESIS OF NANOPARTICLES FROM AGRO-WASTE: A SUSTAINABLE APPROACH

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**Abstract - Nature comprises of many biotic species like plants, algae, fungi, yeast, etc. which are composed of biomolecules. They take part in the formation of nanoparticles with distinct shapes and sizes thereby acting as a driving force for the biosynthesis of nanoparticles with an environmentally benign process. The use of waste materials not only reduces the cost of synthesis but also minimizes energy requirement in comparison to physical or chemical synthesis methods, the need of using harmful chemicals or byproducts, and stimulates 'green synthesis'. Implement of Agro-waste would be unquestionably a strong step towards sustainable development. The nanotechnology seeks its application in all most all area of science and technology, agriculture is no exception to that, yet a lot of development in the field has to happen for establishment of nanotechnology in agro industrial sector as agriculture is known as the back bone of our economy.**

**Keywords— Agro-waste, nanoparticles, green synthesis**

## I. INTRODUCTION

Agriculture contributes a large share to the GDP of our country and according to the Central Statistics Office (CSO), the share of agriculture and allied sectors, was 16.1 per cent of the Gross Value Added (GVA) during 2014–15. However near

about 350 million tonnes of agro-waste is generated every year in India alone and near about 998 million tonnes globally. This includes crop waste, food processing waste, animal waste and also hazardous and toxic waste like pesticides insecticides, herbicides etc. Although there are technologies utilizing this waste to generate energy, the organic waste coming from agriculture remains a challenge, researchers and investigators need to focus on more and more ways to reduce this waste and derive direct and indirect benefits from it for a sustainable growth. Anyhow one of the solutions to this giant pile of problem may be answered by nanotechnology.

In recent years nanotechnology has reached nearly every sector of science ranging from medical, industrial, agricultural, and even house old utilities. And have massively facilitated in evolution of existing technologies. Nanotechnology refers to a field of science where materials ranging from the size of 1 to 100 nm are dealt with. All though in agriculture sector there is a huge gap to be full field, as the use of nanotechnology has been theoretical but it is ongoing and will have significant effect in the areas of food industries, development of new functional materials, products, development and design of methods and instrumentation for food safety and bio-security (Joseph and Morrison, 2006). Nanotech research will definitely aid agriculture in next sage development of genetically modified organism (GMPs), atomically modified organism (AMOs), animal products, pesticides, farming techniques and many more possibilities to



explore with a large potential to revolutionize the agriculture scenario globally.

The synthesis of nanoparticles (NPs) with control over particle size, shape and crystalline nature has been one of the main objectives for potential applications. NPs act as a bridge between bulk materials and atomic or molecular structures. Therefore, they are good candidate for applications including medical, catalysis, electrochemistry, biotechnology, and trace-substance detection (Wen et al., 2011 and Xia et al., 2013). Different synthetic methods have been employed for the preparation of NPs with diverse morphology and size. Although these methods have resulted in superior NPs but still a key understanding of improved manufacturing process is required which could be exploited at the industrial and commercial level to have better built, long lasting, cleaner, safer and smarter products such as home appliances, communication technology, medicines, transportation, agriculture and industries. Therefore, the main focus is to design NPs using environmentally benign approaches. These provide solutions to growing challenges related to environmental issues.

Traditional technologies use a *top-down* approach when constructing materials. Most objects are created starting from a bulk materials and then breaking it into smaller pieces using mechanical, chemical or other forms of energy until they precisely form the desired construction (*e.g.* integrated circuits in microelectronics) (Kawazoe and Meech, 2005). Alternatively, the *bottom-up approach* recognizes that the building blocks of life (enzymes, and other components of each living cell), already act as machines at the nanoscale. Striving for alternative and cheaper pathways for nanoparticle synthesis, scientists contributed to the development of a relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials (Mohanpuria et al., 2008). A great deal of effort has been put into the search for methods utilizing biological systems in order to produce metal nanoparticles at ambient temperature and pressure without requiring hazardous agents and generating poisonous by-products.

Biosynthesis of NPs is a type of bottom-up approach where the main reaction occurring is reduction/oxidation. Various micro-organisms such as bacteria, fungi, and yeasts have been suggested as nanofactories for intra- and extra-cellular synthesis of metals (Singaravelua et al., 2007). The use of plants for nanoparticle synthesis is a comparatively new and under-researched technique. Synthesis of metal NPs using plant extracts is very cost effective, so can be used as an economic and valid alternative for the large-scale production of metal nanoparticles (Huang et al., 2007).

The bioreduction of metal NPs by combinations of biomolecules found in plant extracts such as enzymes, proteins, amino acids, vitamins, polysaccharides, typically

obtained by contact of a broth of plant leaves with metal salts, has been intensively investigated in recent years (Iravani, 2011). The efficient and rapid extracellular synthesis of Ag, Cu and Au nanoparticles using broth extracts of several plants has been reported. For a more exhaustive list of plant species whose extracts have been used for metal nanoparticle production, refer to Narayanan and Sakthivel (2011).

Table 1: Bioaccumulation and biotransformation of nanoparticles

| Species                   | Growth substrate | Element           | Concentration in plant                  | Reaction observed                         | E° (V) SHE | Fraction              | Particle size              | Morphology  | Reference                     |
|---------------------------|------------------|-------------------|-----------------------------------------|-------------------------------------------|------------|-----------------------|----------------------------|-------------|-------------------------------|
| <i>Medicago sativa</i>    | Agar system      | Au                |                                         | $\text{AuCl}_4^- \rightarrow \text{Au}^0$ | 1.0        | Shoots                | 4-40 nm                    | Icosahedral | Gardea-Torresdey et al., 2002 |
| <i>Medicago sativa</i>    | Agar system      | Ag                |                                         | $\text{Ag}^+ \rightarrow \text{Ag}^0$     | 0.80       | Roots, shoots         | 2-20 nm                    | Spherical   | Gardea-Torresdey et al., 2003 |
| <i>Brassica juncea</i>    | Soil*            | Au-Cu<br>Au-Cu-Ag | Au 1120 ppm<br>Au 760 ppm<br>Cu 300 ppm | $\text{AuCl}_4^- \rightarrow \text{Au}^0$ |            | Plant biomass         | 5-50 nm                    |             | Marshall et al., 2007         |
| <i>Brassica juncea</i>    | Soil*            | Ag, Au, Cu        | Ag 730 ppm<br>Au 760 ppm<br>Cu 300 ppm  | $\text{Cu}^{2+} \rightarrow \text{Cu}^0$  | 0.35       | Plant biomass         | 5-50 nm                    |             | Haverkamp et al., 2007        |
| <i>Chilopsis linearis</i> | Agar system      | Au                | 32-179 ppm                              | $\text{AuCl}_4^- \rightarrow \text{Au}^0$ |            | Roots, shoots, leaves | 8 Å (roots), 35 Å (stems), |             | Rodriguez et al., 2007        |





| Species                                                                     | Growth substrate | Element | Concentration in plant | Reaction observed                                                                                                                                                 | E°(V) SHE            | Fraction      | Particle size                                              | Morphology                                 | Reference                    |
|-----------------------------------------------------------------------------|------------------|---------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|---------------|------------------------------------------------------------|--------------------------------------------|------------------------------|
|                                                                             |                  |         |                        |                                                                                                                                                                   |                      |               | 18 Å (leaves)                                              |                                            |                              |
| <i>Sesbania drummondii</i>                                                  | Agar system      | Au      | 5-98 ppm (shoots)      | $\text{AuCl}_4^- \rightarrow \text{Au}^0$                                                                                                                         |                      | Roots, shoots | 6-20 nm                                                    | Spherical                                  | Sharma et al., 2007          |
| <i>Brasica juncea</i>                                                       | Hydroponic       | Ag      | 12.4%                  | $\text{Ag}^+ \rightarrow \text{Ag}^0$                                                                                                                             | 0.80                 | Plant biomass | ~50 nm                                                     | ~Spherical                                 | Harris and Bali, 2008        |
| <i>Brasica juncea</i>                                                       | Hydroponic       | Ag      |                        | $\text{Ag}^+ \rightarrow \text{Ag}^0$<br>$\text{Ag}(\text{NH}_3)_2^+ \rightarrow \text{Ag}^0$<br>$\text{Ag}(\text{S}_2\text{O}_3)_3^{3-} \rightarrow \text{Ag}^0$ | 0.80<br>0.37<br>0.04 | Stems, leaves | 2-35 nm                                                    |                                            | Haverkamp and Marshall, 2009 |
| <i>Brasica juncea</i>                                                       | Hydroponic       | Au      |                        | $\text{AuCl}_4^- \rightarrow \text{Au}^0$                                                                                                                         |                      | Roots, stems  | 2-200 nm                                                   | Spherical Triangular Hexagonal Ecagonal    | Bali and Harris, 2010        |
| <i>Cucumis sativus Helianthus annuus Lolium multiflorum Medicago sativa</i> | Hydroponic <i>   | Au      | 500-2500 ppm           | $\text{AuCl}_4^- \rightarrow \text{Au}^0$                                                                                                                         |                      | Roots, shoots | Variable growth conditions affect size distribution Range: | Spherical Triangular Hexagonal Rectangular | Starne et al., 2010          |

| Species                   | Growth substrate | Element | Concentration in plant                 | Reaction observed                                                                                                                                         | E°(V) SHE | Fraction             | Particle size                                                                                                 | Morphology | Reference                   |
|---------------------------|------------------|---------|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|----------------------|---------------------------------------------------------------------------------------------------------------|------------|-----------------------------|
| <i>Moringa oleifera</i>   |                  |         |                                        |                                                                                                                                                           |           |                      | 1-50 nm                                                                                                       |            |                             |
| <i>Trifolium pratense</i> |                  |         |                                        |                                                                                                                                                           |           |                      |                                                                                                               |            |                             |
| <i>Brasica juncea</i>     | Hydroponic       | Ag, Au  | Ag 0.40% (leaves)<br>Au 0.44% (leaves) | $\text{Ag}(\text{NH}_3)_2^+ \rightarrow \text{Ag}^0$<br>$\text{Ag}(\text{NH}_3)_2^+ \rightarrow \text{Ag}^0$<br>$\text{AuCl}_4^- \rightarrow \text{Au}^0$ |           | Roots, stems, leaves | Ag: 10-30 nm (roots), 4-6 nm (stems); Au: 2-40 nm (roots), 2-100 nm (stems, leaves), 100 nm (leaf cell walls) | Spherical  | Beattie and Haverkamp, 2011 |

Table 2: Typical process for synthesis of Nanoparticles from Agriwastes

| No | Starting material(s)                                         | Reagents/other materials                                                                                                | Procedural highlights                                                                                | Product(s)                                           | Particle size (nm) |
|----|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------|
| 1  | Corn tissues (leaves, roots, stalks, silks, and husks)       | HCl, ethanol solution of tetrabutyl titanate                                                                            | Acid pre-treatment, in-situ growth (impregnation), calcination, crystallization<br>Max. Temp. 550 °C | porous TiO <sub>2</sub> -SiO <sub>2</sub> composites | 13.8 – 20.3        |
| 2  | Rice husk (RH)                                               | HCl, H <sub>2</sub> SO <sub>4</sub> , HNO <sub>3</sub> , CH <sub>3</sub> COOH, NaOH, CaO, NH <sub>4</sub> OH, EDTA, KOH | Acid pre-treatment, combustion, leaching, pyrolysis, sol-gel<br>Max. Temp. 500 °C, 700 °C, 1,000 °C  | Silica                                               | 6                  |
| 3  | RH (from 2 different sources)                                | acetic, citric and phosphoric acids                                                                                     | Leaching, calcination<br>Max. Temp. 650 °C                                                           | Silica                                               | 181.2 – 294.7      |
| 4  | Corn cob                                                     | NaOH, HCl, cetyltrimethyl ammonium chloride (CTMAC), ammonia                                                            | Pyrolysis, sol-gel, calcination<br>Max. Temp. 700 °C                                                 | Silica                                               | 305                |
| 5  | Rice, sylvan horsetail, scouring horsetail and larch needles | HCl, Mg, H <sub>2</sub> SO <sub>4</sub> , HF                                                                            | Hydrolysis, calcination, metallothermic reduction<br>Max. Temp. 700 °C                               | Amorphous silica                                     | 50 – 200           |
| 6  | sugarcane bagasse                                            | H <sub>2</sub> SO <sub>4</sub> , benzene, methanol, acidified NaCl, KOH,                                                | Pulverizing, isolation, acid-hydrolysis<br>Max. Temp. 105 °C                                         | Cellulose                                            | 20-60              |



**Figure 2: Nanotechnology application in agriculture**

#### IV. MINIMIZATION OF WASTE PRODUCT:

Agro waste consists of organic waste as well as hazardous waste like chemical pesticides and fertilizer. Nano materials are being proved to be potential as pollutant removal agent from soil and water as well. Organic particle and pesticides like DDT, chlorpyrifos, malathion etc. can be removed with the aid of nanoparticles. A variety of nanoparticles filters have been used in remediation of waste site in developed countries (Karn et al, 2009).

Nanoscale zero-valent iron is the most widely used for the remediation and apart from this other nanomaterial used in remediation are different metal oxides (for example Zinc oxide applied in arsenic removal treatment), carbon nanotubes, nano enzymes. According to Gilman (2006) technologies like synthetic clay nano-mineral hydrotalcite column and leaching through porous pots or filter candles can be coupled for filtration of water. (T.A. McMurray et al 2006) Has studied and shown that the filters coated with TiO<sub>2</sub> nanoparticles can be used for the photocatalytic degradation of agrochemicals in contaminated water.

The technique of nanofibers from wheat straw and soya hulls for bio-nanocomposite production with the help of nanomaterials produce through engineered plants or microbes (A. Alerndar et al, 2008) was reported at Canadian Universities and Ontario Ministry of Agriculture, Food and Rural Affairs, CA.

A number of applications are expected for food and agriculture uses, including nanosensors, potentially capable of detecting chemical contaminants, viruses, and bacteria; nano delivery systems, which could precisely deliver drugs or micronutrients at the right time and to the right part of the body; as well as nanocoatings and films, nanoparticles, and quantum dots (Bouwmeester et al., 2009). New research also aims to make plants use water, pesticides and fertilizers more efficiently, to reduce pollution and to make agriculture more environmental friendly (Suman et al., 2010).



**Figure 1: Major application of nanotechnology in Agriculture**

#### II. INCREASING EFFICIENCY OF RESOURCE:

The resources are getting over exploited day by day and it has become a matter of concern. Nanotechnology can help in using these resources wisely and durably. This can be achieved by soil improvement by techniques like Zeolite and nano-clay implementation for water or any other agrochemicals assists water infiltration and retention, as it is very porous and have capillary suction activity, it is an excellent amendment for non-wetting and assists water distribution through soil (Prasad et al 2014, <http://www.geohumus.com/us/products.html>).

#### III. INCREASING PRODUCTION RATES AND YIELD:

There is a huge gap between the food supply and demand and to fulfill it is important to increase production from the field. There is lot of development in traditional methods for increases yield like: fertilizer application, chemical and physical pest control techniques, and integrated pest management. But recent studies on application of nanotechnology have brilliantly added the existing technology by not only accelerating their activity but also by increasing their self-life and delivery system.



Hence Nanotechnology is budding and proliferating in agriculture as it shows applications in fields like sustainable and quality agriculture and can enhance the quality of life through improved and rich food for community. The effects on society as a whole will be dramatic (Prasad et al., 2012a).

This review is given in an attempt to focused on one of the sustainable method where in the biosynthesis of various nanoparticle can be mediated through different type of agro waste which includes post harvested waste, weeds plant pathogens and plant pathogens. As recently, the search for cleaner methods of synthesis has ushered in developing bio-inspired approaches. Bio-inspired methods are advantageous compared to other synthetic methods as; they are economical and restrict the use of toxic chemicals as well as high pressure, energy and temperatures (Parashar et al, 2009)

#### V. BIOSYNTHESIS OF NANOPARTICLES THROUGH POST-HARVEST AGRO WASTE

The post-harvest waste is almost 80% of the biomass on the agricultural fields and is often burnt in fields resulting in large amount of green gas emissions, also problems like smog arises, causing serious health impact. Although composting of this for manure production and few for bio fuels production are been practiced this agro waste can also be utilize for the synthesis of valuable nanoparticle. The waste management represents an important challenge in the agri-food based industries and demands an integrated approach in the context of recycling, reuse and recovery (Krishnaswamy.K et al.,2014). Plants in general are well known as bioreactors for their potential of synthesis of nanoparticle. Synthesis of nanoparticle through plant parts is a simple and one stage process ( Figure 3).

**Figure 3: One stage synthesis of nanoparticles**



The synthesis of nanoparticle can be intracellular or extracellular. The synthesis time, growth temperature, pH (He et al) all play important role in size and type of nanoparticle. Finding the right biological method can depend upon a number of variables. Most importantly, the type of metal nanoparticle under investigation is of vital consideration, as in general organisms have developed resistance against a small number of metals, potentially limiting the choice of organism. However synthetic biology; a nascent field of science, is starting to address these issues in order to create more generalized chassis, able to synthesize more than one type of

metallic nanoparticle using the same organism (Edmundson et al. 2014). So far, metal NPs formation in living plants has been observed for gold, silver, copper and zinc oxide. However The ease of extraction and percentage synthesis is making organic synthesis of nanoparticle a matter if great interest.

Nanoparticles thus obtain are further purified and recovered, they are further subjected to Physicochemical characterization like SEM, TEM, DLS, XRD and once the desired shape and size id obtained they are equipped for their applications. Various Agro-waste employed in green synthesis of nanoparticles reported in literature is presented in **Table 3**

**Table 3** Synthesis of nanoparticle using Agro-waste

| Type of Agro-waste                      | Nanoparticle produced | Reference                                            |
|-----------------------------------------|-----------------------|------------------------------------------------------|
| <i>Citrus aurantifolia</i> peel extract | Ag-NPs                | P. Dauthal and M. Mukhopadhyay., 2014                |
| Moringa oleifera                        | Ag-NPs                | Mohammad and Abd El-Rahma., 2015                     |
| Pink guava waste extract (PGWE)         | Ag-NPs and Au-NPs     | Norashikin Ahmad Zamanhur et al.,2012                |
| Sugarcane Bagasse                       | Ag-NPs                | Mishra and Sardar.,2013                              |
| Banana peel extract                     | Au-NPs                | Bankar A et al., 2010 , Preeti D & Mausumi M .,2015, |
| Grape Waste                             | Au-NPs                | Krishnaswamy K et al.,2014                           |
| Cauliflower broth                       | Ag-NPs                | Sridhara et al.,2013                                 |
| Mango peel                              | Au-Nps                | N. Yang et al.,2014                                  |
| Annona squamosal peel                   | Ag-NPs                | Kumar et al.,2102                                    |
| Timber industry waste                   | Ag-NPs                | Devadiga et al., 2015                                |

This cited study suggests that the agro-waste can be potentially employed in synthesis of nanomaterial, but a lot of research is demanded in order to standardize the protocols and methods of synthesis. Utilization of Agro-waste in prospect of nanoparticle production would lead to sustainable development with respect to environment and economy development.



## VI. BIOSYNTHESIS OF NANOPARTICLES THROUGH WEEDS

Weeds are the unwanted plants, herbs or shrubs in an agricultural field. The weeds are usually uprooted and burned. Though many weeds have shown pharmaceutical important drugs properties and are being explored further. Hence the potential of weeds as bioreactors for nanomaterial synthesis cannot be denied and they should be explored more and more for nanoparticles synthesis as weeds mediated green synthesis do not involve shedding of the big green trees it would be more apt for environmental sustainable synthesis of nanoparticles. The table (**Table 4**) below summarizes the recent studies on green synthesis of nanoparticle mediated through weeds.

**Table 4.** WEEDS EXPLORED FOR SYNTHESIS OF NANOPARTICLE

| Weed                            | Nanoparticle synthesized | Reference                                                   |
|---------------------------------|--------------------------|-------------------------------------------------------------|
| <i>Lantana camara</i>           | Cu NPs                   | Majumder et al., 2012                                       |
| <i>Ipomoea carnea</i>           | Ag Nps                   | Abasaheb Ramchandra et al., 2013 , S.U. Ganaie et al., 2014 |
| <i>Kappaphycus alvarezii</i>    | AgNps                    | Ganesan. V et al., 2013                                     |
| <i>Parthenium hysterophorus</i> | AgNps                    | Vyom parashar et al ,2009<br>M.Kalaiselvi et al., 2013      |
| <i>Achyranthes aspera</i>       | AgNPs                    | V. Durga et al., 2014                                       |
| <i>T. cordifolia</i>            | AuNPs                    | Tasneem Abbasi Et al., 2014                                 |
| <i>Gloriosa superba</i> L       | CuONPS                   | H . Raja Niaka et al, 2014                                  |

## VII. PLANT PATHOGENS IN BIOSYNTHESIS OF NANOPARTICLES

Recently the “Green chemistry” has attracted a lot of all the investigators across the world as it is environmental friendly and cost effective. Plants are been proved to be the best bioreactors for the synthesis of nanomaterial, however other organism like Bacteria, Fungi and viruses are also being explored for special size and stabilization.

**Bacteria:** Prokaryotes have received most attention in biosynthesis of nanoparticles (Mandal et al 2006), due to the reason that they can be manipulated more easily than eukaryotes at genetic levels for increasing the amount of nanoparticle synthesis. Nanoparticles like silver, gold, magnetite, FeS and quantum dots of cadmium sulphide (CdS), lead sulphide (PbS) and Zinc sulphide (ZnS) can be biosynthesized.

**Fungi:** There has been a shift from bacteria to fungi to be used as natural “nanofactories” owing to ease downstream processing, easy handling (Mandal et al.,2006) as they are able to produce large amount of enzymes. However understanding the mechanism of synthesis of nanoparticle is important for getting a control over desired shape and size. Few investigators have reported pathogenic mediated synthesis of various nanoparticles.

**Plant viruses:** Viruses are made up of Single or double stranded DNA/RNA as genomes which are packed in a protein cote and are potential of carrying the nucleic acid molecule from one host to another. They themselves are a form of naturally occurring nanomaterials/ nanoparticles, especially the spherical and icosahedra viruses. (Hoglund, 1968) has reported the smallest virus till date of only 18 nm in diameter known as satellite tobacco necrosis virus (STNV). The recent review by (Young et al, 2008) on plant viruses as bio template for nanomaterial and their application give a clear picture of their employment in nanotechnology. **Table 5** briefs about the plant pathogens explored for synthesis of nanoparticles

**Table 5:** SYNTHESIS OF NANOPARTICLE USING PLANT PATHOGENS.





| Name of organism                       | Nanoparticle synthesized | Synthesis location            | Reference                 |
|----------------------------------------|--------------------------|-------------------------------|---------------------------|
| <b>Fungus</b>                          |                          |                               |                           |
| <i>Macrophomina phaseolina</i> (Tassi) | Ag-NPs                   | Extracellular                 | Chowdhury et al., 2014    |
| <i>Alternaria alternata</i>            | Ag-NPs                   | Extracellular                 | M. Gajbhiye et al., 2009  |
| <i>F.oxysporum f.sp.cubense</i> (Foc)  | Ag_NPs                   | Extracellular                 | Dhandhukia et al., 2012   |
| <i>Neurospora crassa</i>               | Pt-NPs                   | Intracellular & Extracellular | Sanghi R et al., 2011     |
| <i>Fusarium oxysporum</i>              | CdS-NPs                  | Extracellular                 | 41                        |
| <i>Verticillium sp.</i>                | Au-NPs                   | Intracellular                 | Sanghi R et al., 2009     |
| <b>Bacteria</b>                        |                          |                               |                           |
| <i>Rhodospseudomonas capsulate</i>     | Au-NPs                   | Extracellular                 | He S et al., 2007         |
| <i>Pseudomonas aeruginosa</i>          | Au-NPs                   | Extracellular                 | Narayanan KB et al., 2010 |

## VIII. CONCLUSION

The field of nanotechnology is fast-growing and, beyond the results already achieved in related sciences, full of promising prospects, some of which are probably not yet imagined, with applications in medicine, consumer goods, heavy industry, information and communication technologies, optoelectronic devices, and environmentally-friendly energy systems. Nanotechnology has the potential to revolutionize agriculture and food systems. Even though the agro-industry is just beginning to explore its applications, nanotechnology has exhibited great potential with new tools for the molecular treatment of diseases, weed control and enhancing the ability of plants to absorb nutrients (Jha *et al.*, 2011).

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