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Green synthesis of silver nanoparticles (AgNPs): Agricultural applications and future vision

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Abstract

Silver nanoparticles (Ag NPs) have gained huge attention and popularity in the scientific world due to their intriguing physical, chemical and antimicrobial properties. Ag NPs can be synthesized by physical, chemical and biological processes. Since there is pressing need for eco-friendly, and sustainable synthetic method, extensive research is taking place about the green synthesis of Ag NPs using plants. Biosynthesized Ag NPs show excellent biocompatibility and antibacterial property and hence have wide applications in agriculture and other fields. In view of this, we have reviewed here the use of plants or their extracts for the synthesis of Ag NPs, their characterization, and effect of physicochemical parameters on the synthesis. This review underlines the applications of biosynthesized Ag NPs in the agriculture sector with a short note on its future prospects.

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Introduction

Nanotechnology is a newly developing branch having great potential to enhance the quality of life through its applications in various domains like agriculture, therapeutics, drug delivery, cosmetics, biosensors and so forth. A nanoparticle is a miniature molecule having a size range in between 1 to 100 nm. Nanoparticles are of incredible scientific enthusiasm as they are adequately a scaffold between mass materials and nuclear or atomic structures. As compared to the bulk material, nanoparticles show novel physical, chemical, electrical, mechanical, thermal, optical, dielectric and biological properties. Due to nano size, they exhibit extremely larger surface area in contrast with their volume, which lowers their melting temperature by hundreds of degrees than bulk material (Balaguru and Jeyaprakash, 2010). Conversion of metals into nanoparticles exhibits different striking colors due to the effect of Surface Plasmon Resonance (SPR) (Rivera *et al.*, 2012). Chemical properties of metals are also seen to be changed at the nanoscale, for example, conversion of the inert metal into catalyst (platinum) (Boysen and Muir, 2011). As a result of these striking properties, nanoparticles are proved to be lucrative as compared to the bulk material.

Synthesis of nanoparticles having desired properties is one of the most challenging areas of research in nanotechnology. Researchers from various disciplines are engaged in exploring the best protocol for the synthesis of stable and monodispersed nanoparticles. Till today numerous techniques/methods for nanoparticles synthesis have been reported. Biological synthesis of nanoparticles using plants and microbes is developed to discomfiture the limitations of conventional synthetic methods such as physical and chemical procedures. A biological method uses nontoxic, biocompatible, and environment-friendly substrates and is relatively easier synthesis process under ambient conditions (Singh *et al.*, 2015, Nadaf and Kanase, 2016). Synthesis of nanoparticles using plant extracts is rapid but can form polydispersed nanoparticles.

The application of nanoparticles is another important emerging area of nanoscience and nanotechnology. Nano-sized metal nanoparticles have been widely studied for their applications in various fields including electronics, photonics, catalysis, medicine, food industry. Till now many metal nanoparticles have been synthesized such as silver, gold (Aljabali *et al.*, 2018; Iyer and Panda, 2018), copper (Kimber *et al.*, 2018), platinum (Syed and Ahmad, 2012; Raut *et al.*, 2018), zirconium oxide (Park *et al.*, 2005), zinc oxide (Sangeetha *et al.*, 2011; Bhuyan *et al.*, 2015), titanium dioxide (Sundrarajan and Gowri, 2011; Rajakumar *et al.*, 2012). Among various metals, AgNPs are the material of choice of researchers due to their remarkable properties such as broad-spectrum antimicrobial activity, surface enhanced Raman scattering, catalytic activity, chemical stability and nonlinear optical behavior (Krutyakov *et al.*, 2008). Broad-spectrum bactericidal and fungicidal activity of Ag NPs has made them extremely popular component in the field of medicine, consumer products, and agriculture.

Agriculture is the backbone of the national economy in developing countries; nowadays it is facing major challenges, including changing climate, urbanization, sustainable use of natural resources and environmental issues like runoff and accumulation of pesticides and fertilizers (Ditta, 2012). Furthermore, there is continued stress on agricultural resources due to increasing population and future demand for food will increase tremendously while natural resources such as land, water, and soil fertility are limited (Ditta, 2012). With the advancement of technology, nanotechnology can help the agriculture sector by detecting and controlling plant diseases, pest control, increasing livestock production. Use of nano products and nano systems in the agricultural sector may improve the crop productivity.

Several reviews are published on the green synthesis of nanoparticles, though critical analysis of enormous information on part wise plant-mediated Ag NPs synthesis with special emphasis on agricultural application is still missing.

Therefore, this review article represents a systematic overview of green synthesis of Ag NPs by various plant extracts and describes the mechanism of nanoparticles synthesis. Various analytical techniques utilized to characterize nanoparticles are also discussed. Finally, this review highlights the applications of biosynthesized AgNPs in the agriculture sector with short notes on its future prospects.

Silver nanoparticles

Since ancient times silver and its salts have been used as an antimicrobial agent (Nadaf and Kanase, 2015) through its use was discontinued due to side effects (Singh *et al*., 2015). However, almost a decade, nanoscale silver made a remarkable comeback due to its amazing properties (Tolaymat *et al*., 2010). Ag NPs are nanoparticles having a size range between 1 nm and 100 nm in size. They are one of the widely studied nanoparticles. Different sizes and shapes can be considered and modified for various types of applications. Ag NPs are safe to use since they have low toxicity as Ag has no interaction towards living organisms during preparation (Navarro *et al*., 2008). Spherical shaped Ag NPs are used commonly, but the octagon and thin sheets Ag NPs have also popularity in their uses (Graf *et al*., 2003). The distinctive characteristics of Ag NPs make them a tool to investigate via research and evaluate their potential effectiveness, harmfulness. Various shapes of Ag NPs have been manufactured relying upon the demand of the application. Ag NPs have broad-spectrum antimicrobial properties so can be used in biomedical field, medical devices, textile (Toh *et al*., 2017), water purifiers, cosmetics, food industry (Chaudhry and Castle, 2011) in wound-healing, therapy, DNA processing, and pharmaceuticals (Zhang *et al*., 2012) and so on.

Synthesis of silver nanoparticles

There are various methods used by the scientists to manufacture NPs. Two broad approaches of NPs manufacture are 'Top-down' and 'Bottom-up' manner. In the top-down approach, the bulk molecule is broken down into the tiny atomic level.

On the other hand, the bottom-up approach uses to synthesize NPs by accumulating them. Evaporation-condensation and laser ablation are the most important physical approaches to synthesize AgNPs. The most widely recognized mechanism for the manufacture of silver NPs is a reduction of chemicals by organic or inorganic agents. Sodium citrate, ascorbate, and sodium borohydride ($NaBH_4$) are used as reducing agents in various aspects (Zielińska *et al*., 2009). Metallic Ag NPs are synthesized by reducing the Ag^+. As the Ag NPs have worldwide recognition, due to its wide applicability scientist are focusing on synthesizing them by biological methods than the chemical method.

Green synthesis of silver nanoparticles

Plants are able to reduce metal ions, both on their surface and in various organs and tissues remote from the ion penetration site (Makarov *et al*., 2014) and are known to have great potential in heavy metal accumulation and detoxification (Yang *et al*., 2005). Considering these potentials, researchers are paying attention to the utilization of plants for metal nanoparticles synthesis. Green synthesis of nanoparticles using plants has emerged as a facile and viable alternative to more complex, toxic chemical synthetic procedures to obtain Ag NPs. Utilizing plants to synthesize Ag NPs is safe since chemical methods use environmental hazardous reagents and physical methods are quite expensive compared to the other two methods.

Now a day's environmental safety issue is a big concern to conduct a research. So, scientists prefer green synthesis because it is less toxic, eco-friendly, rapid (Mohapatra *et al*., 2015) and bulk production is possible (Mittal *et al*., 2015). Additionally, plant-mediated synthesis of Ag NPs is a much cheaper way since it does not require cell culture maintenance (Shankar *et al*., 2004). When Ag NPs are chemically synthesized, three main components are required silver salt (e.g., $AgNO_3$), a reducing agent (e.g., $NaBH_4$), and stabilizing or capping agent (e.g., polyvinyl alcohol) for controlling the size of nanoparticles and preventing their aggregation (Ledwith *et al*., 2007).

In contrast, green synthesis does not require any additional reducing agent or stabilizing agent. The biological way of synthesizing Ag NPs is a smart approach used by the researchers as the biosynthesized nanoparticles are more appropriate for medical and pharmaceutical applications (Abdel-Halim *et al.*, 2011). Selection of solvent medium and selection of plant species are the most important issues which must be considered in green synthesis of nanoparticles. There are two main factors which influence the size, shape, and stability of nanoparticles, namely, the concentration of the plant extract/metabolite and the substrate (metal ions) concentration (Rajan *et al.*, 2015).

Different plant part extracts such as leaves, fruits, flowers, barks, gums, latex, peels, roots etc. of various species have been reported to be successfully used in metal nanoparticles synthesis. A general strategy to synthesize Ag NPs using plant extract is shown in Figure 1.

Many plants are reported to facilitate AgNPs synthesis are listed (Table 1) and are discussed in brief in this review. Gardea-Torresdey *et al.* (2002), Shankar *et al.* (2003), and Ankamwar *et al.* (2005) have worked extensively on the biosynthesis of metal nanoparticles from various plant sources. Gardea-Torresdey *et al.* (2002) are the first to report the formation of gold and Ag NPs inside a living plant. They are pioneers for nanoparticle synthesis using plant extracts.

Table 1. Green synthesis of AgNPs using different plant species.

Plant name	Plant parts	Size of the NPs in nm	Shape of the NPs	References
Emblica officinalis	Fruit	10-20	Spherical	Ankamwar *et al.*, 2005
Aloe vera	Leaf	~16	Spherical	Chandran *et al.*, 2006
Solanum torvum	Leaf	~14	Spherical	Govindaraju *et al.*, 2010
Artemisia nilgirica	Leaf	70-90	Square	Vijayakumar *et al.,* 2013
Bamboo	Leaf	~100	Spherical	Yasin *et al.*, 2013
Azadirachta indica	Leaf	4-18	Spherical	Nazeruddin *et al.*, 2014
Solanum xanthocarpum berry	Fruit	4-18	Spherical	Amin *et al.*, 2012
Dillenia indica	Fruit	40-100	Spherical	Singh *et al.*, 2013
Chrysanthemum morifolium	Flower	20-50	Spherical	He *et al.*, 2013
Achillea biebersteinii	Flower	~13	Spherical	Baharara *et al.*, 2014
Boswellia ovalifoliolata	Bark	30-40	Spherical	Ankanna *et al*, 2010
Boswellia ovalifoliolata, Shorea tumbuggaia	Bark	NA	Spherical	Savithramma *et al.*, 2011
Pinus eldarica	Bark	10-40	Spherical	Iravani and Zolfaghari, 2013
Ficus benghalensis	Bark	~40	Spherical	Nayak *et al.*, 2015
Anogeissus latifolia	Gum	~6	Spherical	Kora *et al.*, 2012
Astragalus gummifer	Gum	~14	Spherical	Kora and Arunachalam, 2012
Jatropha curcas	Latex	~14	Spherical	Bar *et al.*,2009
Hevea brasiliensis,	Latex	2-15	Spherical	Guidelli, *et al.*, 2011
Jatropha curcas, Jatropha gossypifolia, Pedilanthus tithymaloides, and *Euphorbia milii*	Latex	62-263	Spherical	Patil *et al.,* 2012
Ficus sycomorus	Leaf Latex	≤20 nm ≤100	Irregular	Salem *et al.*, 2014
Musa spp. (Banana)	Peel	NA	NA	Bankar *et al.*, 2010
Citrus spp. (Orange)	Peel	~8	Spherical	Kahrilas *et al.*, 2013
Lepidium draba	Root	20-80	Spherical	Benakashani *et al.*, 2017

The spectacular success in this field opened the prospect of developing "green synthesis" methods for metal nanoparticles with tailor-made structural properties using plant-based starting materials. Chandran *et al.*, (2006) reported the synthesis of Ag NPs using *Aloe vera* plant extract. They reported that reduction of silver ions by *Aloe vera* extract led to the formation of spherical Ag NPs of 15.2 nm ± 4.2 nm size. The wastage part of saffron (*Crocus sativus* L.) aqueous extract can also be used to prepare Ag NPs (Bagherzade *et al.*, 2017). The green synthesized Ag NPs were characterized by several techniques such as UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM) and X-ray diffraction (XRD) analysis.

Synthesis using leaf extract

The synthesis of AgNPs using the leaf extract of *Solanum torvum* was reported (Govindaraju *et al.*, 2010). These AgNPs were spherical with antibacterial activity against some pathogens. *Artemisia nilagirica* leaf extract mediated synthesis of AgNPs was demonstrated by Vijayakumar *et al.* (2013).

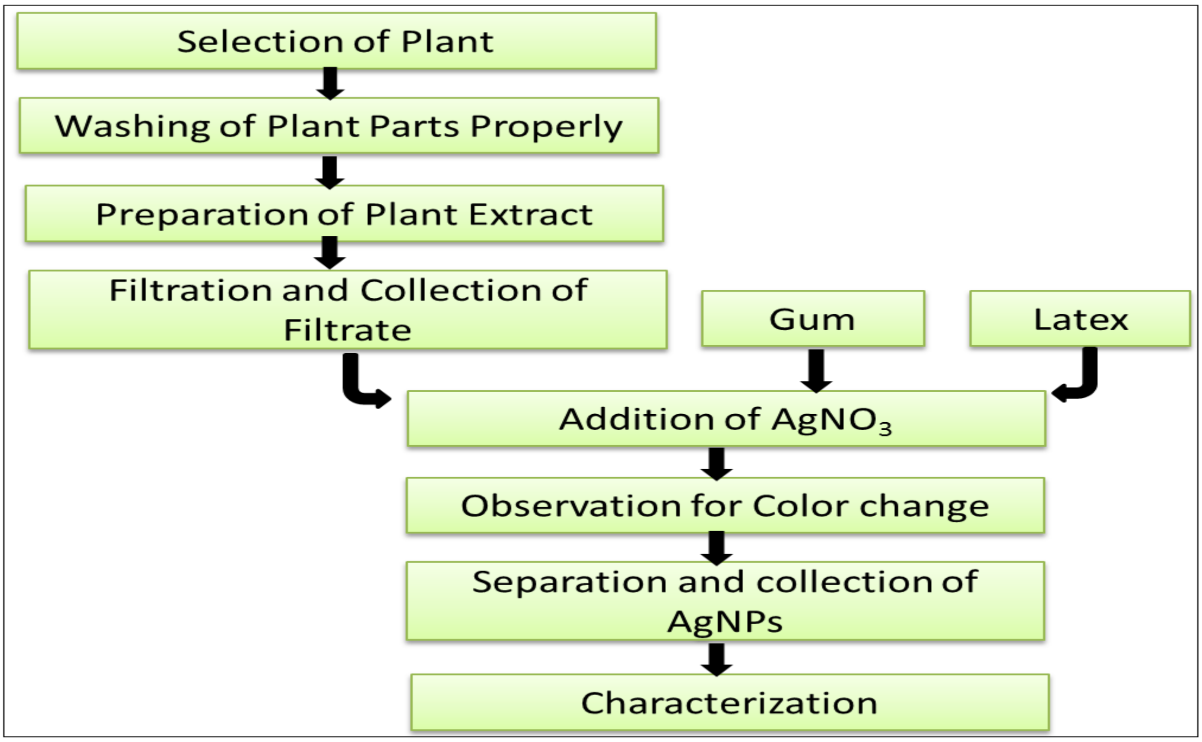

Fig. 1. General approach to prepare AgNPs from plant material.

The morphology of the AgNPs was determined by SEM and the average diameter of the particles was determined as 70 to 90 nm.

They observed that the size of square Ag NPs increased as the dosage of *A. nilagirica* leaf extract increased to 20 ml. Yasin *et al.* (2013) reported the Ag NPs synthesis using Bamboo leaf extract. Bamboo leaves are known to be rich in various phytochemicals like flavonoids, phenolic acids, and lactones. Bamboo leaves are also a common source of derivative compounds of phenolic acids, coumaric lactones and flavonoids like; homoorientin, orientin, isovitexin, vitexin, naringin-7-rhamnoglucoside, rutin, quercetin, luteolin, tricin, cafffeic acid, chlorogenic acid and phydroxy coumaric acid (Lu *et al.*, 2005, Lu *et al.*, 2006).

Nazeruddin *et al.* (2014) demonstrated the AgNPs synthesis by leaf extract of *Azadirachta indica* without using any surfactant or external energy. With this method rapid synthesis of nanoparticles was observed to occur; *i.e.* reaction time was 1 to 2 hr as compared to 2 - 5 days required in microbial synthesis.

They observed that the nanoparticles formed were of different sizes and particle size was found to be 4.74 nm, 8.17 nm, 14.23 nm and 18.98 nm and the mean size of about 11.5nm which lies in the nano range. These nanoparticles were found to be polydispersed. (Nazeruddin *et al.*, 2014). Swamy *et al.*, (2015) reported the synthesis of the AgNPs using a methanol leaf extract of *Leptadenia reticulata*.

Synthesis using fruit extract

The fruits have also been proved to be an excellent candidate for the synthesis of nanoparticles as well. The synthesis of Papaya fruit extract mediated AgNPs have been demonstrated (Jain *et al.*, 2009). Edison and Sethuraman (2012) investigated the potential of aqueous extract of *Terminalia chebula* for the synthesis of AgNPs. The synthesized AgNPs were found to be 25 nm in diameter. On the exposure of silver salt to extract of *Emblica officinalis,* the stable AgNPs of size range 10 to 20 nm were found be synthesized (Ankamwar *et al.*, 2005). Bioreductive potential of *Piper longum* fruit in synthesis of AgNPs has been described by Reddy *et al.* (2014). They revealed from FTIR data that the capping of the phytoconstituents, probably polyphenols from *P. longum* fruit extract is stabilizing the synthesized nanoparticles**.** The green synthesis of spherical AgNPs with dimensions 4 to 18 nm were observed using a methanol extract of *Solanum xanthocarpum* berry (Amin *et al.*, 2012). The extract *Dillenia indica* fruit, an edible fruit which is widely distributed in the foothills of the Himalayas was also used to synthesize AgNPs. The reduction of silver ions to AgNPs by this extract was completed within 166 h. The particle size of these AgNPs was ranging from 40 to 100 nm (Singh *et al.*, 2013). Other fruits such as *Ananas comosus* (Ahmad and Sharma, 2012), *Lantana camara* (Kumar *et al.*, 2015), *Malus domestica* (Umoren *et al.*, 2014)*, Averrhoa bilimbi* (Isaac *et al.*, 2013), *Crataegus douglasii* (Ghaffari-Moghaddam and Hadi-Dabanlou, 2014) etc. have also been reported to synthesize AgNPs.

Synthesis using flower extract

Flowers are also proved as good candidates for Ag NPs synthesis. Many researchers challenged the flower extract with $AgNO_3$ and found exciting results. He *et al.* (2013) synthesized the water soluble Ag NPs by treating silver ions with *Chrysanthemum morifolium* Ramat. extract at room temperature. TEM analysis revealed the formation of spherical nanoparticles with an approximate size of 20 to 50 nm. Baharara *et al.* (2014) reported simple, environmentally friendly, low-cost synthesis of Ag NPs using the *Achillea biebersteinii* flower extract. The average particle diameter as determined by TEM was found to be 12 ± 2 nm.

The synthesized Ag NPs from the flowers of *Achillea biebersteinii* extracts were observed to be very stable in the solution even one month after their synthesis. *Cassia fistula* flower extract has also been reported to synthesize Ag NPs by acting as reducing and capping agent. FTIR analysis revealed that the reduction and capping of AgNPs is due to the presence of alkanoids in flower extract (Remya *et al.*, 2015). The phenolic compounds are capable of functioning as reducers of metal ions. *Pinus merkusii* contains phenolic and flavonoid compound like pinosylvin, pinosylvin monomethyl ether, dimethyl ether pinosylvin, and pinocembrin (Wijayanto *et al.*, 2015). Azkiya *et al.* (2018) demonstrated the synthesis of Ag NPs using cone flower extract of *Pinus merkusii.* They studied the effect of extract concentration and reaction time on nanoparticles synthesis. It was observed that increased concentration of the flower extract leads to increased production of the nanoparticles (Azkiya *et al.*, 2018). Synthesis of monodisperse Ag NPs using *Rhododendron dauricum* flower extract has also been reported. The optimum extract to metal salt ratio is required for the synthesis of symmetrical nanoparticles. They investigated that 2ml of *Rhododendron dauricum* flower extract diluted with 100 ml water indicates the formation of relatively monodispersed particles. The stability study showed that the synthesized nanoparticles have been quite stable for a few months (Mittal *et al.*, 2012).

Synthesis using bark extract

The plant bark extract has been successfully used in synthesis of Ag NPs. Bark extract is also rich in phytochemicals such as phenols, flavonoids and antioxidants. The well dispersed Ag NPs of size 30 to 40nm were synthesized using the stem bark extract of *Boswellia ovalifoliolata*. The reduction of silver ions to Ag NPs by this stem bark extract was completed within 10 min (Ankanna *et al*, 2010).

Savithramma *et al.* (2011) reported the synthesis of the AgNPs using bark extract of the Endemic medicinal plant *i.e. Boswellia ovalifoliolata* and *Shorea tumbuggaia*. Bark extract *Boswellia ovalifoliolata* synthesized nanoparticles within 10 min, whereas *Shorea tumbuggaia* took 15 min to synthesize. When *Pinus eldarica* bark extract was exposed to silver ions, the color of the reaction mixture turned to yellowish brown and then to dark brown, which indicated the formation of AgNPs (Iravani and Zolfaghari, 2013). On the exposure of silver nitrate solution to *Ficus racemosa* bark extract synthesis of the AgNPs took place within 30 min (Velayutham *et al.*, 2013). Bark extracts of bark extracts of *Ficus benghalensis* and *Azadirachta indica* were also found to have the potential to produce Ag NPs. The average particle size was around ~40 and ~50 nm respectively, for nanoparticles synthesized from *F. benghalensis* and *A. indica* and were formed within 30 min (Nayak *et al.*, 2016). *Zizyphus xylopyrus* bark extract (Maria *et al.*, 2015) and *Callicarpa maingayi* bark extract (Shameli *et al.*, 2012) were also reported to synthesize Ag NPs.

Synthesis using gum and latex

Gum is a sap, a polysaccharide-based material frequently associated with certain plant species, especially woody plants, particularly under the bark or as a seed coating. A survey of earlier literature suggests that plant-based exudates gums can be successfully used as reducing agents for the synthesis of Ag NPs. Kora *et al.*, 2012 reported gum ghatti (*Anogeissus latifolia*) mediated synthesis of Ag NPs. Synthesis under optimized conditions resulted in the formation of nearly monodispersed spherical AgNPs of size around 5.7 ± 0.2 nm. In another report, Kora and Arunachalam (2012) described AgNPs synthesis using Tragacanth (*Astragalus gummifer*) gum. They studied the role of gum concentration and reaction time on the synthesis of nanoparticles. By regulating the reaction conditions, spherical nanoparticles of 13.1 ± 1.0 nm size were produced. Based on FTIR analysis, it has been suggested that both hydroxyl and carbonyl groups of gum are involved in the synthesis AgNPs. Cashew gum is natural plant product exudates from *Anacardium occidentale* L. tree. Quelemes *et al.* (2013) developed the method for synthesis of AgNPs using this gum. Synthesis was carried out within 60 min in an open glass reactor with magnetic stirring, in temperature-controlled water bath at 78 ± 2 °C. Recently, Velusamy *et al.* (2015) also investigated the autoclave assisted synthesis of Ag NPs using gum extract of neem (*A. indica*). TEM and atomic force microscopy (AFM) analysis revealed the average diameter of the synthesized nanoparticles was to be <30 nm.

Latex is a stable emulsion of polymer microparticles, found as a milky fluid in angiosperms. Plant latex has proved to be a good natural source for synthesis of AgNPs. Latex of *Jatropha curcas* can be used as an effective capping as well as reducing agent for the synthesis of Ag NPs. 3% of latex solution of *J. curcas* was found to be the optimum one for the present green synthesis Ag NPs. The synthesis involved the heating of reaction mixture (latex and aqueous silver nitrate solution) at 85°C with constant stirring for 4 h in oil bath. They observed bimodal size distribution of Ag NPs (Bar *et al.*, 2009). Natural rubber latex extracted from *Hevea brasiliensis* has also been explored for its Ag NPs synthesis potential. The synthesis using this latex resulted in the formation of well dispersed particles with a diameter from 2-15 nm which is revealed after TEM analysis (Guidelli, *et al.*, 2011). Patil *et al.* (2012) reported one-step solvent-free synthesis of AgNPs using *Euphorbiaceae* plant latex. They investigated the synthesis of Ag NPs using four different plant species viz. *J curcas, J gossypifolia, Pedilanthus tithymaloides*, and *Euphorbia milii*. The average size of the plant latex synthesized nanoparticles was found to be larger i.e. between 62 to 263 nm (Patil *et al.*, 2012). Recently bioreduction potential of latex and leaf extract of *Ficus sycomorus* has also been reported. It was observed that formation of Ag NPs started rapidly after 2 h of incubation. The biosynthesized Ag NPs were ellipsoidal in shape, sometimes spherical with few agglomerated particles (Salem *et al.*, 2014).

Synthesis using peel extract

Naturally available agricultural and food industry wastes have also been investigated for the synthesis of Ag NPs. Boiled, crushed, acetone precipitated, air-dried banana peel powder was used for reducing silver nitrate. The synthesis of Ag NPs occurred within 3 min at 80ºC upon addition of banana peel extract powder to silver nitrate solution. It was believed that functional groups associated with banana peel polymers like pectin, cellulose, and hemicellulose as well as the proteinaceous matter may be involved in reducing the silver salt to aggregated Ag° (Bankar *et al*., 2010). One step microwave assisted AgNPs were successfully synthesized in 15 min using orange peel extract. The TEM analysis revealed nanospheres having a mean diameter (with standard deviation) of 7.36 ± 8.06 nm. Based on gas chromatography-Mass spectroscopy (GC-MS) data, it is suggested that aldehydes present in the orange peel extract may be responsible for the improved AgNPs synthesis (Kahrilas *et al*., 2013).

Synthesis using root extract

Many researchers have reported the synthesis of AgNPs using the plant root extract. *Lepidium draba* root have been used to fabricate AgNPs by Benakashani *et al*. (2017). They obtained AgNPs of size ranging between 20-80 nm. They reported that smaller size Ag NPs can be fabricated by increasing the concentration of root extract of *L. draba*. Spherical silver nanoparticles with a size of 30–55 nm was synthesized by Root extract of *Morinda citrifolia* (Suman *et al.,* 2013). Velmurugan *et al*. (2014) reported the synthesis of AgNPs of size 10-20 nm, using *Zingiber officinale* root extract, which acted as a reducing and capping agent.

Mechanism of Silver Nanoparticles synthesis

Various hypotheses have been proposed to elucidate the synthesis of Ag NPs, though conclusive and conspicuous mechanism still to be disclosed. Based on several investigations here we have described bacteria mediated synthesis of Ag NPs (Naik *et al*. 2002; Jha and Prasad 2010; Gaidhani *et al*. 2013; Singh *et al.,* 2015), wherein the role of bacterial genes, enzymes and reducing agents, and peptides have been widely manifested. Accumulation of silver (Ag) mainly occurred in two different stages: (i) during non-specific attachment to the cell surface, and (ii) intercellular accumulation (Shakibaei *et al*. 2003). The silver binding machinery of bacterial cell play leads to cellular uptake of Ag^+ ions, wherein three major genes such as *silE, silP* and *silS* play a pivotal role in Ag NPs synthesis (Parikh *et al.,* 2008). Silver binding gene *silE* encodes silver binding protein responsible for Ag^+ ion binding, Consequently, Ag^+ ions are reduced to malic silver nuclei or seed nanoparticle by silver binding machinery (Parikh *et al*., 2008). These Ag NPs appear to be formed in different shapes and sizes. Sintubin *et al*. (2009) evidenced that lactic acid bacteria, *Lactobacillus* spp. can reduce Ag^+ ions into silver nanoparticles. *Lactobacillus* spp. can generate smaller size (10–20 nm) AgNPs.

Roles of enzymes reducing agents and peptides have been marked widely. NADP-dependent enzymes particularly nitrate reeducates (NR) reported playing important role in Ag NPs synthesis (Kalimuthu *et al*., 2008). The enzyme NR gains the electron from NADH and oxidizes to NAD, consequently it brings the further reaction and reduce the Ag^+ ions into nanosilver. Naik *et al*. (2002) reported that peptides receive major attention for synthesis and stabilization of Ag NPs, peptides can interact with perform Ag^+ nanoclauster in solution. As a consequence, generates a reducing environment that leads to reduce Ag^+ ions and the formation of polydispersed Ag NPs.

Hence, from the above-mentioned facts the synthesis of Ag NP by biological approach is due to the presence of genes, proteins, enzymes, coenzymes, reducing agents which capable of donating electron for the reduction of Ag^+ ions to aggregated Ag°. The active ingredient responsible for the reduction of Ag^+ ions may vary based on organisms/synthesize approaches used. Srikar *et al*. (2016) provided a schematic diagram showing the silver ion reduction, agglomeration, and stabilization to form a particle of nano size is shown in Figure 2.

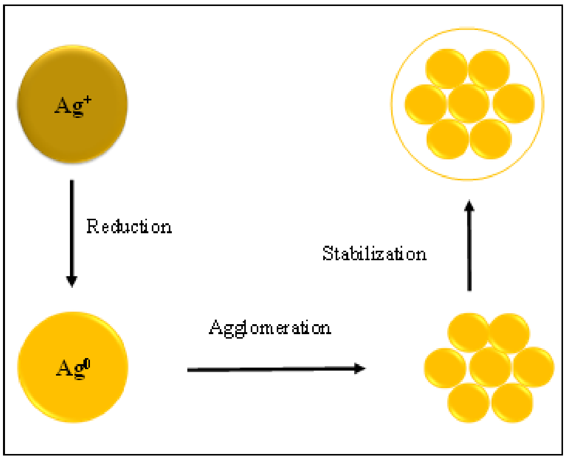

Fig. 2. A schematic diagram for synthesis mechanism of Ag NPs (modified from Srikar *et al.,* 2016).

Factors affecting Silver Nanoparticles synthesis

The major physical and chemical parameters that affect the synthesis of Ag NPs are pH, reaction temperature and time, metal ion concentration, extract contents and concentration, duration of the reaction and agitation. These parameters largely affect the size, shape, and the morphology of the Ag NPs (Kora *et al.*, 2010). It is observed that the basic pH of reaction mixture shows a rapid growth rate, good yield with mono dispersity, and enhanced reduction process (Khalil *et al.*, 2014; Das *et al.*, 2012). Small and uniform sized nanoparticles were synthesized by increasing pH of the reaction mixture (Ortega-Arroyo et al., 2013; Sathishkumar et al.,2012; Dehnavi *et al.*, 2013). However, very high pH (pH > 11) was associated with the drawback of formation of agglomerated and unstable Ag NPs (Tagad *et al.*, 2013). The Reaction conditions like time of stirring and reaction temperature are important parameters. Temperatures up to 100°C were used by many researchers for AgNPs synthesis using biopolymers and plant extracts. Plant extract concentration also has a significant effect on the size and morphology of the synthesized Ag NPs. It is reported that the particle size increases with an increase in the ratio of leaf extract and quercetin separately to the $AgNO_3$ solution (Jain and Mehata, 2017). Increasing volume of Wolfberry fruit extract also resulted in the formation of larger particles (Dong *et al.*, 2017). Increasing metal ion concentration also affects the reduction process. As the silver nitrate concentration increases, SPR peak also increases, which indicates the faster rate of bioreduction with increased concentration of precursor salt (Zaki *et al.*, 2012). The hike in peak intensities with increasing silver nitrate concentration was also observed by Ghosh *et al.* (2012).

Separation and characterization of Silver Nanoparticles

Centrifugation is the commonly used technique to collect the pellet of synthesized Ag NPs. The pellet of Ag NPs is generally dried to get it in powder form (Sadeghi *et al.*, 2015). Most of the characterization techniques require sample in solid form, however some require liquid form. The characterization is a critical step because size, shape, surface charge, structure, etc. are major factors to concern about the functional properties of Ag NPs. Preliminary detection of formation of Ag NPs in a laboratory is usually done by visual observation for color change. Colorless reaction medium turns to yellowish brown or brown during synthesis of Ag NPs (Kumar *et al.*, 2014, Moodley *et al.*, 2018, Shaik *et al.*, 2018). Some common characterization techniques include UV-Vis Spectra, XRD, Field Emission Scanning Electron Microscopy (FESEM) and SEM, TEM, Energy Dispersive spectroscopy (EDS), and dynamic light scattering (DLS). Noble metals have optical properties which absorbs strongly in the visible region due to Surface Plasmon Resonance. Such optical properties are identified by a UV visible spectrophotometer (Jain and Mehata, 2017). XRD is the powerful and non-destructive basic technique used for fingerprint characterization of crystalline materials and thin films. It also gives information about phase purity, crystallinity, crystal structure and percent phase composition. FESEM and SEM is an ideal technique used to study the topographic details of the surface of the nanoparticles. (Vijayaraghavan *et al.,* 2012, Ortega-Arroyo et al., 2013, Sathishkumar *et al.*, 2012). By using this technique high resolution surface imaging can be done, hence proved useful in the field of nanomaterials science. TEM is a powerful technique used to investigate the morphological details, particle size distribution of nanomaterials. TEM also furnish diffraction patterns of the specimen which helps to comprehend the detailed crystal structure analysis of the sample. This diffraction analysis can be used to find out size dependent changes in the lattice parameters as well as defects in the sample (Kulkarni, 2015). Energy Dispersive analysis of X- Ray is a useful analytical technique used for the elemental analysis of the particles. DLS is a most popular technique used to determine the size distribution profile of particles in liquid phase. Dynamic light scattering is also known as photo correlation spectroscopy. This technique can measure the nanosized particles (Lin *et al.*, 2009) and band gap energy (Zhang *et al.*, 2012). Zeta potential values indicate the stability of synthesized Ag NPs. Thermo-Gravimetric Analysis (TGA) is used to find the effect of $AgNO_3$ on the organic composition of Ag NPs to find out the amount of organic material in synthesized Ag NPs (Morales-Sánchez *et al.*, 2011) and predict the thermal stability of Ag NPs (Kora *et al.*, 2010). Inductive Coupled Plasma (ICP) analysis is performed to analyze the concentration and conversion of AgNPs (Song and Kim, 2009).

Applications of Silver Nanoparticles in Agriculture

In most of the countries, agriculture is the backbone of development and the majority of people depend on agricultural products. Scientists are trying to find a way to feed the rapidly growing world population over 7 billion at present. Currently, nano agriculture focuses on specific farming by using nano size particle having unique biomimetic properties to boost productivity. The nanoscale particles ensure target specification, involuntary, multifunctional capabilities (Nair *et al.*, 2010). Nanotechnology is the rapid advance science at present and has both scientific and industrial applications. Scientists are giving the effort to find out their potential application in drug delivery, cancer therapy as well as in agriculture (Gu *et al.*, 2011). So phytonanotechnology focus on developing 'smart crops'. In agriculture system, nanotechnology has a demand for pest protection and nutritional enrichment. This reduces the frequent use of chemical fertilizers in conventional farming. The scope of nanoparticles in agriculture is shown in Figure 3.

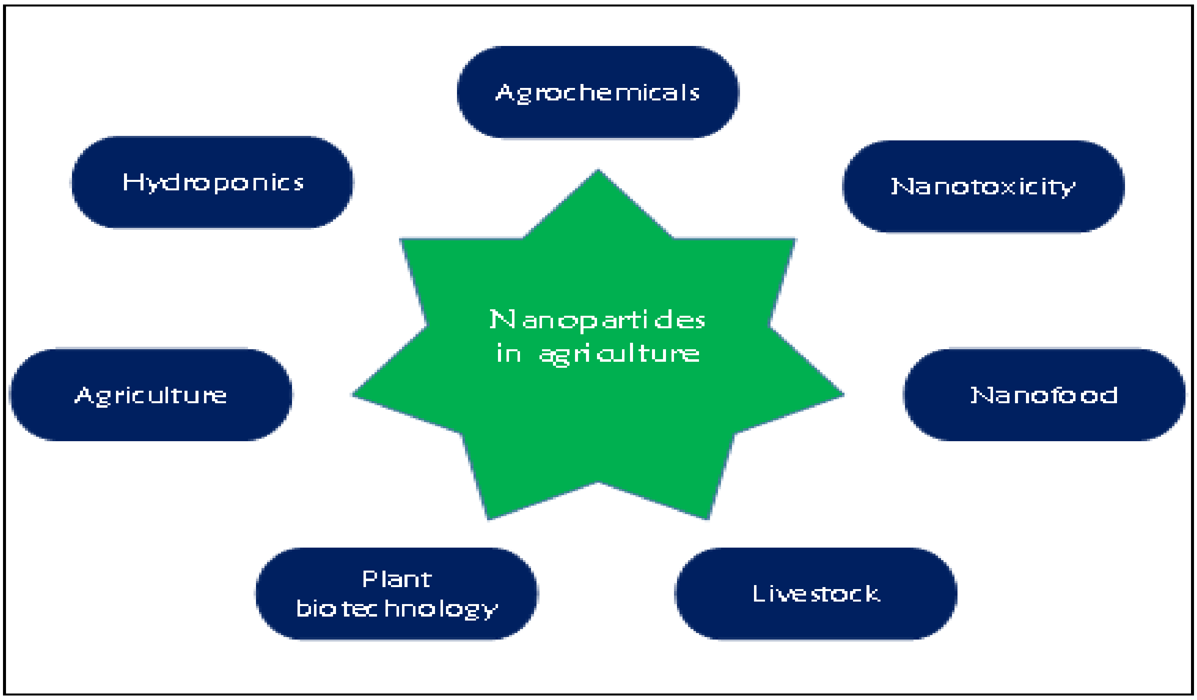

Fig. 3. Uses of nanoparticles in agricultural.

In plant biotechnology, research and development in nano size particles facilitate and edge the upcoming genetic modification in crops. At this moment, the use of Ag NPs in agriculture is mostly theoretical. Researchers think that in the near future the world agriculture will largely depend on the nanotechnology because they are getting significant positive results in the laboratory-based applications. The applied field of Ag NPs in agriculture is depicted in Figure 4.

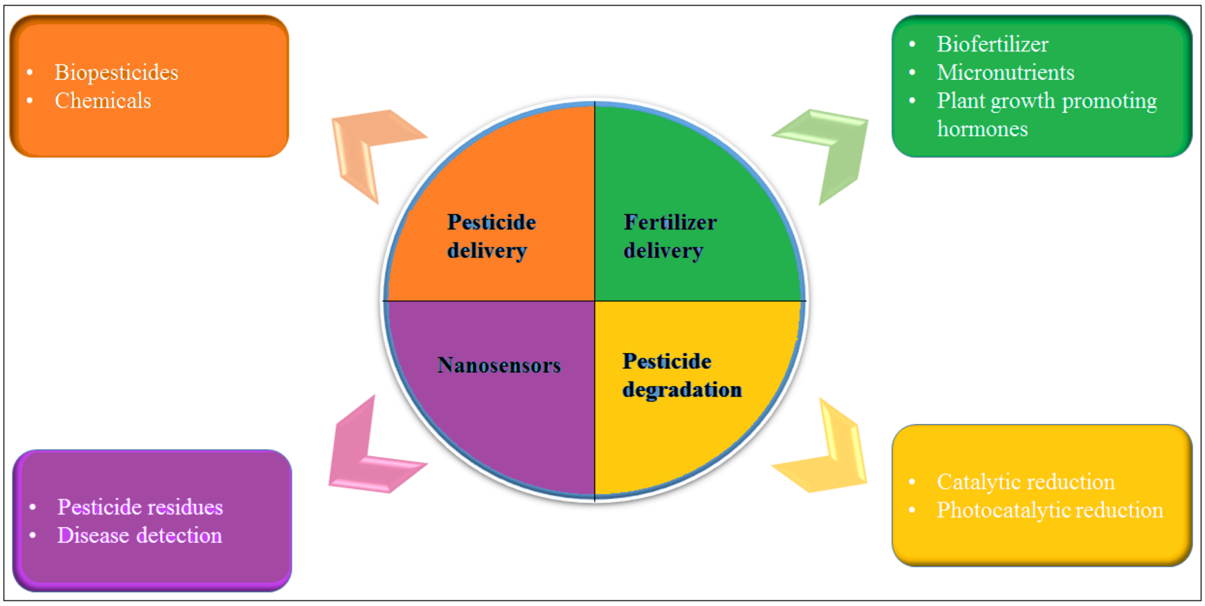

Fig. 4. The applied field of Ag NPs in agriculture.

Size, surface charge and other factors play a crucial role to show some extraordinary characteristics of the Ag NPs. Spherical shaped Ag NPs are commonly used now. Its enormous greater surface area permits the synchronization of a vast number of ligands. Ag NPs have well-known recognition for their antioxidant, antibacterial, antifungal, anti-viral and anti-inflammatory properties. By controlling nutrients, this nanotechnology acts as a crucial part in the production of agricultural products (Mukhopadhyay, 2014). Greenhouse experiment of *Triticum aestivum*, *Brassica juncea* and *Vespertilio sinensis* show positive results on plant growth after treating them by Ag NPs. Root and shoot length were taken as plant growth parameter (Mehta *et al.*, 2016).

Silver nanoparticles in plant disease management and crop protection

The application of Ag NPs as antimicrobial agents is because of its broad spectrum activity and multiple modes of inhibitory action against pathogens. The well dispersed and stabilized, colloidal Ag NPs are more adhesive on bacterial and fungal cell surface; hence act as better bactericide and fungicide. Many research groups have reported the plant disease control with the application of Ag NPs. Management of diseases of food crops and fruits is economically important. Ocsoy *et al.* (2013) have developed DNA-directed silver NPs grown on grapheme oxide and studied the antibacterial activity against *Xanthomonas perforans*, a causative agent of bacterial spot in Tomatoes. They observed that the application of DNA-directed Ag NPs grown on grapheme oxide at 100 ppm on Tomato transplant in a greenhouse experiment significantly reduced the severity of bacterial spot disease compared to untreated plants. Ouda (2014) demonstrated the inhibitory action of Ag NPs against two plant pathogens; *Alternaria alternata* and *Botrytis cinerea* under different concentrations in growth medium. The antimicrobial activity of plant extract is due to the presence of secondary metabolites such as tannin, saponin, and glycosides (Polash *et al.*, 2017). Kim *et al.* (2012) also investigated the *in vitro* antifungal activity of Ag NPs against nineteen different plant pathogenic fungi. Colloidal AgNPs also show antifungal activity against very common disease rose powdery mildew caused by *Sphaerotheca pannosa* in both greenhouse and outdoor Rose plants (Kim *et al.,* 2008). It results in leaf curling, early defoliation, and reduced flowering. Through a chemical reaction, double capsulized nano-silver was prepared to aid in reducing agent and stabilizers (Kim *et al.,* 2008). These highly stable and very well dispersive aqueous solutions eliminate unwanted microorganisms in planter soils and hydroponics systems. Elamawi *et al.* (2018) also determined the antifungal activity of AgNPs synthesized by *Trichoderma longibrachiatum*, against nine fungal isolates: *Aspergillus alternate*, *Fusarium verticillioides, Fusarium moniliforme, Aspergillus flavus, Aspergillus heteromorphus, Penicillium glabrum, Penicillium brevicompactum, Helminthosporium oryzae,* and *Pyricularia grisea*.

Bacterial diseases are one more cause of significant loss in crop yield worldwide. Ag NPs are proved to be active against plant pathogenic bacteria. Al-Askar *et al.* (2013) revealed that Ag NPs have high antibacterial activity against *Erwinia cartovora, E. amylovora, Dickya chransanthemi, D. dianthicale, Pectobacterium wasaibiae, P. atrosepticum* and *P. wasaibiae* than generic antibiotics. They also studied the antifungal activity of Ag NPs against pathogenic fungi viz. *Fusarium oxysporum, Alternaria alternata* and *Aspergillus flavus* and found promising results.

Silver nanoparticles in plant growth enhancement

Ag NPs have been used in agriculture to increase the crop yield by enhancing the seed germination and plant growth. The growth response of plants to Ag NPs is dosage dependent and could have a positive or negative effect. Exposure of plants to specific concentrations of Ag NPs could promote plant growth as compared to non-exposed plants, whereas higher and lower concentrations could have an inhibitory effect on plant growth. (Kaveh *et al.*, 2013, Geisler-Lee *et al.*, 2012). Sharma *et al.* (2012) examined the growth response of Ag NPs concentrations used (0, 25, 50, 100, 200 and 400 ppm) in *Brassica juncea* seedlings. A 50- ppm treatment has been determined to be optimal with a positive effect on fresh weight, root and shoot length, and a vigor index of seedlings. Almutairi and Alharbi (2015) also examined the effect of Ag NPs dosage on the seed germination of three plant species: corn, watermelon and zucchini. It was found that germination rates of these three plants were enhanced in response to AgNPs.

Pest management

Nanotechnology in pest management includes the formulations of nanomaterials-based pesticides and insecticides. Use of Ag NPs in insect pest management have been reported. Goswami *et al.* (2010) studied the applications of Ag NPs in the control of rice weevil and grasserie disease. Ag NPs treated stored rice remained uninfected even after 2 months of treatment, so it is suggested that Ag NPs can also be used as an excellent seed protecting agent (Goswami *et al.*, 2010).

Food industry

Post-harvest management, including the preservation of agricultural produce is one of the important branches of agriculture. Previous studies have reported that Ag NPs based antimicrobial packaging plays an important role in increasing the shelf life of fresh fruits and vegetables. An *et al.* (2008) evaluated the effect of Ag NPs-PVP coating on preservation of green asparagus. They observed that coating of Ag NPs PVP slowed down the weight loss, ascorbic acid and total chlorophyll, reduced the color changes in the skin of asparagus, inhibited the increasing of the tissue firmness, the growth of microorganism and increased the shelf-life of asparagus by about 10 days at 2^{o}C. Similarly, Mohammed Fayaz *et al.* (2009) also reported the preservation of vegetable and fruits using biosynthesized Ag NPs. They prepared Ag NPs incorporated sodium alginate films and studied its antibacterial activity, measured weight loss (%) of fruits and vegetables, and performed sensory analysis. They observed that Ag NPs incorporated sodium alginate film retained its antibacterial activity against both Gram-positive and Gram-negative organisms.

The minimum weight loss was observed in Ag NPs incorporated sodium alginate film coated carrots and pears compared to sodium alginate coated and uncoated control. The color, appearance, texture and taste of Ag NPs incorporated sodium alginate film coated carrots and pears were found to acceptable up to 10 days of storage as compared to uncoated control and sodium alginate coated carrots and pears. Low-density polyethylene (LDPE) polymer matrix containing Ag NPs was studied to preserve and extend the shelf life of stored barberries (Valipoor *et al.*, 2013). Nano packaging with AgNPs-LDPE successfully maintained the sensory, physicochemical and physiological qualities of barberry and strawberry fruits at a higher level compared with normal packaging with polyethylene bags. Similar effects were obtained in case of Ag NPs-LDPE packages for the preservation of orange juice (Emamifar *et al.*, 2010).

Conclusions and Future prospects

In the past two decades Ag NPs have been extensively studied because they have displayed antimicrobial properties and various other exciting characteristics. Wide ranging applications of Ag NPs have encouraged researchers to synthesize Ag NPs and to know more about these alluring tiny particles. Plants serve as one of the promising candidates for the biosynthesis of Ag NPs as compared to other biological entities. They offer an eco-friendly, cheap, time saving, non-toxic way to achieve Ag NPs. The extracts of various medicinal plant parts, fruit wastes are able to reduce silver ions faster that bacteria and fungi. Many reports have been published about the syntheses of Ag NPs using plant extracts which is already discussed in earlier sections. There is still a need for commercially viable, economic route for large scale production of Ag NPs. By optimizing different physicochemical parameters, synthesis of nanoparticles having desired properties can be achieved. Mechanism of synthesis of Ag NPs using plant extracts is not yet fully understood. In order to have better control over synthesis process, further extensive studies revealing the precise molecular mechanism of formation of AgNPs by biological methods are required. Nanotechnology is a promising technology with the ability to create massive changes in agricultural systems. It is becoming progressively important for the agribusiness. Ag NPs have made their place in '*agriculture*' by exhibiting exceptional characteristics. They are successfully used to increase crop yield, protect the crop from bacterial, fungal infections/diseases and pest attack/infestation. Additionally, Ag NPs are employed in the form of 'nano-packages' to increase the shelf life of fresh agricultural produce like fruits and vegetables. Future research must explore the role of plant metabolites in Ag NPs synthesis, synthesis using purified single metabolites rather than crude extracts, experimentation with process and regulation of physicochemical conditions to obtain various other shapes such as triangle, cube, and hexagon than spherical. Research must be focused on assessment of Ag NPs impact on biotic and abiotic factors of the environment and human health before mass production and use of agricultural applications. As well as, detail investigation for actual field applications is needed.

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