Green Synthesis of Silver Nanoparticles using aqueous extract of *Lycopersicon esculentum* L. var. PKM 1

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ABSTRACT

Green synthesis is an ecofriendly and cost effective procedure for large scale production of Silver nanoparticles. In the present study, silver nanoparticles were synthesized from the aqueous leaf powder extract of *Lycopersicon esculentum* L. var PKM 1. Characterization of silver nanoparticles at optimum condition was done using SEM analysis. This showed the presence of silver nanoparticles at a higher range in tomato plants treated with mixture of organic fertilizer and biofertilizer rather than the control plant. EDAX analysis showed the presence of various elements at different concentration levels. The elements present in the control tomato plant were Carbon, Oxygen, Silver, Chlorine and Gold. Apart from these elements, the mixture of organic fertilizer and biofertilizer treatment showed the presence of Silicon at 2% level. The percentage of silver in tomato plants treated with mixture of organic and biofertilizers was found to be higher (9%) when compared to the control plant (4%). The percentage of Gold was equal (2%) in both control plants and plants treated with mixture of organic and biofertilizers. The presence of silver in the tomato plants treated with mixture of organic and biofertilizer shows that the plant could be used for the production of particles at nano scale that has a wide range of application in industry.

Key Words: Biofertilizers, *Lycopersicon esculentum* L., Organic, Silver nanoparticles, SEM

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INTRODUCTION

Nanotechnology is the study and application of small object which can be rapid growing in the field of science that combines engineering with physics, chemistry and biology and removes the traditional boundaries between them (Ray *et al.*, 2009). The term "Nanotechnology" was given by Norio Taniguchi of the Tokyo Science University in 1974. A nanometer is one billionth (10^{-9}) of a meter and nano refers to a size scale between 1 nanometer (nm) and 100 nm.

It has been very useful in improving the growth, yield and health of fruit crops and its most important advantage has been in the form of Nano Fertilizers (NFs), accounting for the majority of research in this field.

Nanomaterial have properties that have made them extremely useful in biosensing, biological labeling, catalysis, antibacterial activity, antiviral activity, drug delivery, antioxidant applications, DNA sequencing and gene therapy in the recent year (Bharathi *et al*., 2018). Mostly metallic and non-metallic nanoparticles are widely recognized. The non-metallic nanoparticles are carbon, silicon, nitric oxide, chitosan, fullerenes and grapheme oxide, some of the metallic nanoparticles include cobalt, titanium, aluminium oxide, copper, silver, gold, palladium, magnesium, manganese oxide, platinum and zinc oxide. Among these metallic nanoparticles, silver is one of the most commercialized nano-material with five hundred tons of silver nanoparticles production per year (Larue *et al*., 2014). There are growing concerns over the potential adverse impact of nanoparticles to the environment.

Nano-fertilizers have novel properties that enhance the growth and yield of the plant by supplying one or more nutrients, whereas, nanomaterial-enhanced fertilizers improve the performance of conventional fertilizers. The effect of nanoparticles varies from plant to plant and depends on their mode of application and size. Nanosilver is applied in textiles, home water purification system, medical devices, cosmetics, electronics and household appliances. It has strong bactericidal and broad spectrum antimicrobial activities and also reduces various plant diseases caused by spore producing fungal pathogens (Nair *et al*., 2010).

There are many techniques of synthesizing nanoparticles, and they can be largely described as ‘wet or dry’ processes. Wet method of synthesis are frequently referred as “bottom up” synthetic method, since nanoparticles are assembled atom by atom through a process of nucleation while dry method of synthesis known as “top down” method, since they embroil breaking down bulk compound to nanoparticles (Rai, 2016). The synthesis of nanoparticle using naturally occurring reagents such as sugars, biodegradable polymers, plant extracts and microorganisms as reductants and capping agents could be considered attractive for nanotechnology (Ahmed and Ikram, 2015). Numerous methods have been exploited for the synthesis of nanoparticles, however many research works aim to apply a biological green nano technique for the synthesis of silver nanoparticles using plant extract, because it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer product. These nanoparticles are characteristically categorized by their size, surface area, shape and dispersity nature. The common procedures implemented in their characterization are UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM) and X-ray diffraction (XRD).

Chitosan capped AgNPs is an essential class of nanomaterial for a range of medical applications that may have probable risks to human health, which have been documented through studies on the genotoxicity and cytotoxicity of AgNPs. In *Allium cepa* assay, the genotoxic potentials of green synthesized AgNPs with the occurrences of chromosomal aberrations have been reported (Yekeen *et al*., 2017).

Objective of the study

1. To synthesize silver nanoparticles and characterize them in the test plant using Scanning Electron Microscope

MATERIALS AND METHODS

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACT

Preparation of leaf extract

Two samples were taken for testing silver nanoparticle synthesis. About 5g of control plant and tomato plants treated with mixture of organic and biofertilizers were taken for the extract. The samples were transferred into 250 ml conical flask containing 100ml of distilled water and boiled at 60^0C for half an hour. The extract was then filtered through Whatmann No 1 filter paper to remove the particulate matter and to get a clear solution.

Preparation of Silver nitrate solution

1mM silver nitrate solution was freshly prepared in distilled water.

Synthesis of silver nanoparticles

One mM aqueous solution of silver nitrate was prepared and used for the analysis of silver nanoparticles. One ml of plant extract was added to 20 ml of 1mM aqueous solution of silver nitrate ($AgNO_3$) in a sterile conical flask. The solution was heated for few seconds. Later, the reduction of silver ions was observed by a change in the colour of the solution from yellow to brown.

Characterization of Silver Nanoparticles

The characterization of silver nanoparticles was carried out by different techniques such as SEM, UV-Vis and FTIR analysis.

SEM Analysis

Scanning Electron Microscopy is a commonly used method for characterization of silver nanoparticles. Thin film of sample was prepared on a carbon coated grid by placing a very small amount of sample on the grid, then it was allowed to dry and examined under SEM.

EDAX analysis

The sputtering of the sample was done using SC7620 Sputter Coater unit under the nitrogen atmosphere. A small strip of carbon tape was stuck on aluminium stub and a pinch of sample was placed on carbon tape. The elemental analysis and distribution of the particles were carried out using EDAX with SUTW-SAPHIRE model detector.

RESULTS AND DISCUSSION

Green synthesis of silver nanoparticles using control and combined organic and biofertilizer treatment were tabulated with SEM image.

SYNTHESIS OF SILVER NANOPARTICLES

For the synthesis of the silver nanoparticles, colour change was noted by virtual observation in tomato leaf extract incubated with aqueous solution of silver nitrate (Plate 1). The solution was stirred for 2 min. The reduction process Ag+ to AgO nanoparticles was followed by the color change of the solution from light brown and then brownish red colour, this exhibit the formation of silver nanoparticles. There is increase in intensity of absorption peaks after regular intervals of time and the colour intensity increased with the duration of incubation.

Farghaly and Nafady (2015) have utilized the leaf extract of Rosemary for the biosynthesis of silver nanoparticles (AgNPs). The extract is used as both reducing as well as stabilizing agent.

They have shown that the green synthesis of AgNPs from Rosemary leaves is inexpensive and eco-friendly. They have also studied the effect of AgNPs on the growth of wheat and tomato plants.

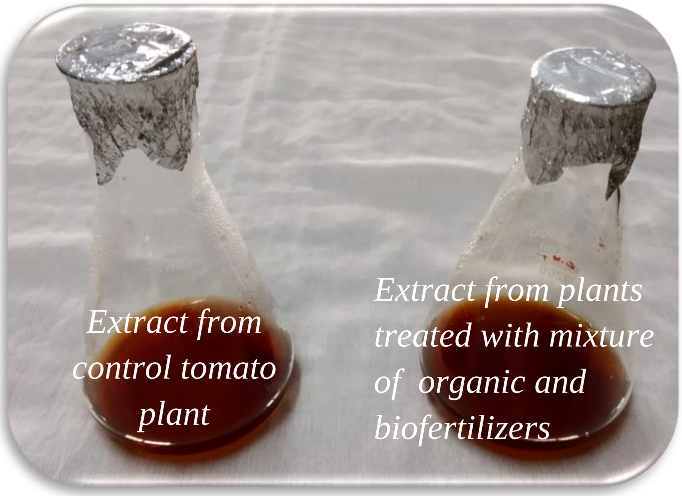

Plate 1: Photograph showing synthesized AgNPs in solution

Characterization of Silver nanoparticles

Generally, nanoparticle synthesis involves physical, mechanical and chemical methods. But these are very expensive and harmful (Mali *et al*., 2018). So, with an aim to develop a clean, nontoxic and eco-friendly technologies, various biological sources has been used for nanoparticle formation. The presence of amino groups, proteins, carbohydrates and carboxylic groups play a vital role in the formation of nanoparticles. Synthesized nanoparticles are important in different fields and so could benefit mankind.

Nanoparticles are generally characterized by their size, shape, surface area and dispersity (Jiang *et al*, 2009). The common techniques of characterizing nanoparticles are as follows:

SEM image

SEM was used to view the morphology and size of silver nanoparticle. SEM image showed the high density nanoparticle synthesized by tomato plant extract and were relatively spherical in shape. This confirmed the development of silver nanostructures.

SEM image (Plate 2) of the sample of control tomato plant confirms the existence of very small and uniformly spherical nanoparticles. It showed that the silver nanoparticles (AgNPs) were spherical in shape with an average size between 13.16 nm to 33.49 nm. This SEM HV is 25kV that implies stability and activate more conductivity in the view field of 2.08μm.

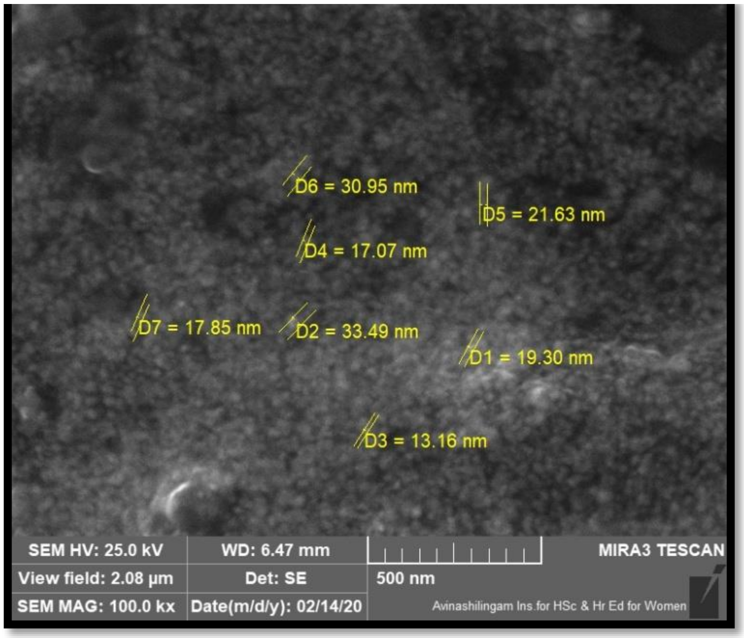

Plate 2: SEM image of the sample of control tomato plant

The SEM image (Plate 3) of tomato plant treated with mixture of organic and biofertilizers showed the nanoparticle in the range of 14.03nm -64.22 nm. The SEM HV is 30.0 kV that implies spherical in shape and high conductivity. This size of particle confirms the presence of nanoparticle.

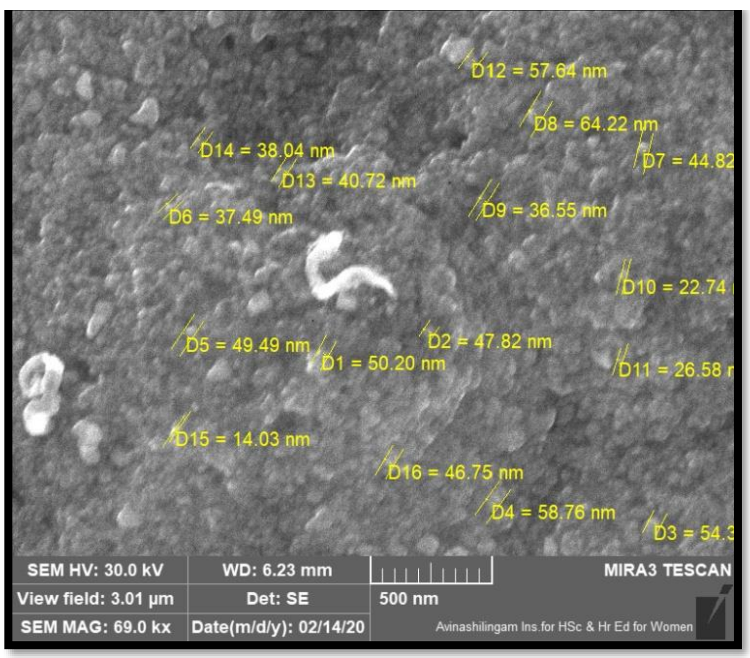

Plate 3: SEM image of tomato plant treated with mixture of organic and biofertilizers

EDAX

Analysis through Energy Dispersive X-ray(EDX) spectrometers confirmed the presence of Elemental silver signal of the silver nanoparticles. The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in KeV. Identification lines for the major Emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified (Fig 1 ; Table 1).

Table 1 Elements present in *Lycopersicon esculentum* L. var PKM 1 of Control plant

Element	Weight%	Atomic%	Elements present in percentage
C	47.96	70.32	62%
O	21.81	24.00	20%
Ag	16.22	2.65	4%
Cl	4.64	2.30	6%
Au	1.37	0.61	2%

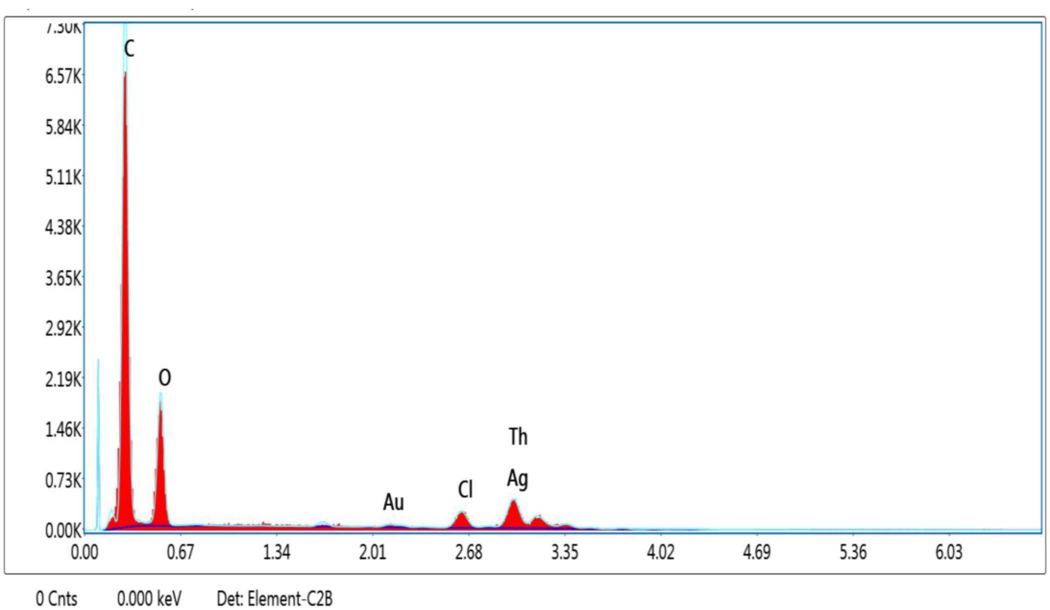

Fig: 1 EDAX spectrum recorded showing sharp peak between 2.8 and 3.0 keV confirming the presence of silver in the sample of *L. esculentum* L. var PKM 1 of Control plant

Table 2 and Fig 2 showed the presence of silver ions and the amount of silver present is the sample. This indicates the reduction of silver ions to elemental silver.

Applications of Nanotechnology in agriculture can prove to be a boon to mankind. It uses the agricultural inputs more effectively and reduces the by-products that could harm the environment as well as human health (Bhagat *et al*., 2015). Logeswari *et al*. (2015) have studied the synthesis of nanoparticles from commercially available plant powders. Use of plant materials for nanoparticle biosynthesis is considered to be a Green technology, since it does not involve any harmful chemicals and it could be used immensely in medical field for their efficient antimicrobial activity.

Tomato is an important vegetable used in our day-to-da life. It can be used as a salad and for various food preparations like juice, soup, sauce, ketchup or puree. The processing by food industry for various products produces large amount of waste at different stages (Many *et al*., 2014) that could be effectively used to produce eco-friendly nanoparticles.

Table 2 Elements present in *Lycopersicon esculentum* L. var PKM 1treated with mixture of organic and biofertilizers

Element	Weight%	Atomic%	Elements present in percentage
C	59.58	73.03	65%
O	26.56	24.45	16%
Ag	10.45	1.43	9%
Cl	2.10	0.87	5%
Au	1.04	0.08	2%
Si	0.28	0.15	2%

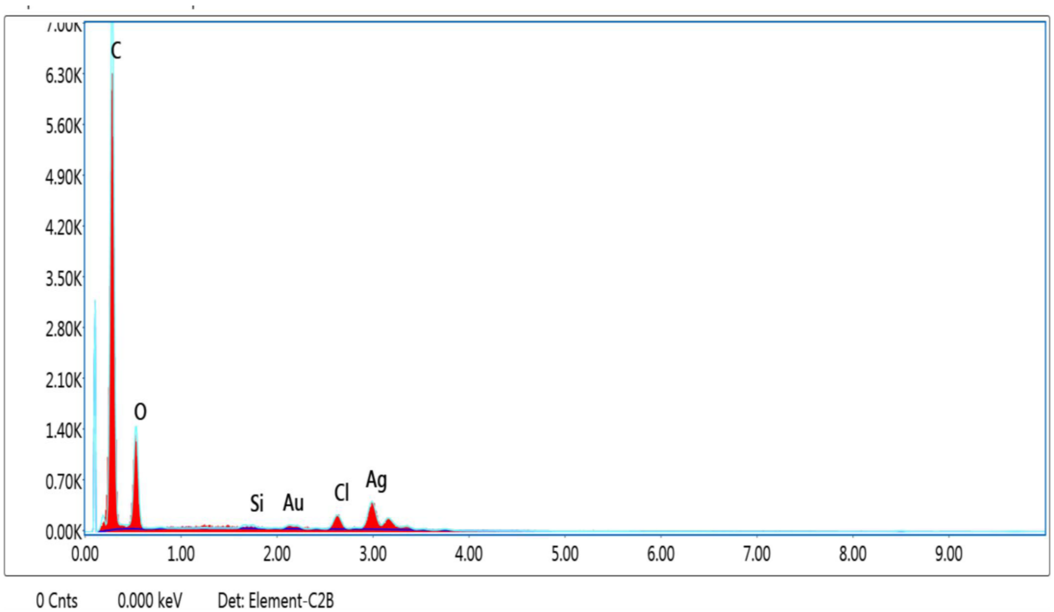

Fig:2 EDAX spectrum recorded showing sharp peak between 2.8 and 3.2 keV confirming the presence of silver in the sample of *L. esculentum* L. var PKM 1treated with mixture of organic and biofertilizers

Green synthesis of nanoparticles is an eco-friendly and economical approach (Sithara *et al*., 2017). Kholoud *et al*. (2010) have studied the synthesis and applications of AgNPs and found that nano-size particles (< 100 nm dia.) are gaining more attention currently for their wide range of application in various industrial fields.

Logeswari *et al*. (2015) have utilized silver nitrate solution for the synthesis of AgNPs from plant extracts.

Nanotechnology due to the production of materials at nano scale level is emerging as a growing field with its application in Science and Technology (Albrecht *et al*., 2006).

The present study on the green synthesis of Silver Nanoparticles is an initiative to understand its presence in tomato plant that could be further utilized for drug development.

Conflict of Interest:

No conflict of interest

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