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Synthesis of green nanobiofertilizer using silver nanoparticles of *Allium cepa* extract Short title: Green nanofertilizer from *Allium cepa*

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Abstract
The present study involved the green synthesis of onion silver nanoparticles (AgNPs) from the onion extract as a reducing agent to develop nanobiofertilizer. Characterization of AgNPs was done by Fourier transform infrared (FTIR) spectroscopy, UV-Visible spectrophotometer and Scanning Electron Microscopy (SEM). UV–vis spectroscopy confirmed the formation of AgNPs by observing the typical surface plasmon resonance peak at 470 nm. The possible functional groups of AgNPs were identified by FTIR analysis. SEM studies revealed that AgNPs were cubical shaped with an average particle size range from 10 to 30 nm. The both the fresh weight and vigor were higher in plants treated with 15 ml/l synthesized nanobiofertilizer compared to control. The synthesized nanobiofertilizer from onion extract is an effective fertilizer for brinjal and tomato plants. The use of such types of nanobiofertilizer can reduce the excessive use of chemical fertilizers, environmental pollution and helps in reduction of farm management costs.

Keywords: *Allium cepa*, silver nanoparticles, nanobiofertiizer, onion

Introduction
Onion *(Allium cepa* L.) is one of the most widely cultivated crop plants all over the world and its bulb is used as both food and medicine. Onions possess strong, characteristic flavors and aromas, which have made them important ingredients of various food items. Onions contains various minerals *viz.* calcium, iron, manganese, magnesium, phosphorus, potassium, zinc, selenium, flouride and iron. Also, it is rich in carbohydrates, proteins, sodium, potassium and phosphorus [1]. The mineral nutrients play a very important role in plant growth and development. Generally, the application of mineral nutrients increases growth, yield and quality of crops. Potassium content in onion peels is about 234 mg of the fruits and it is the most important element that is used as fertilizer. It is essential for promoting the plant vigor, resistance to pest and disease, help in fruit growth, involved in regulating several enzymes in plant [2]. Every macronutrient has its own character and is therefore involved in different metabolic processes of plant life. The signs of potassium deficiency in plants can be seen as the older leaves have brown veins. Moreover, specific types of flavonoids found in onion include quercetin and anthocyanin. The quercetin is a strong antioxidant among the phenolic compounds having physiological and biochemical roles in plants; played protection against salinity in tomato [3]. In recent, to get higher production, farmers are utilizing more chemical nutrients. However, the problems associated with chemical nutrients supplementation in soil are leads to pollution of ground water after harvest. Also, it decorate the soil structure [4], by decreasing the organic matter content leads to nutrient imbalance and soil acidification [5]. Moreover, it has negative impact on microorganism activities. Besides of this, it is not readily available to farmers over the year and high in cost. Other problems include most inorganic fertilizer in the soil is gradually depleted by crops [6]. Recently, huge revolution concerning the utilization of agriculture waste as organic fertilizer was growing over the last years. Natural organic components can be obtained from plant wastes which are transferred to compost rich by the nutritious organic matter to be returned to the soil for fertilization.

In recent term "green synthesis" has been coined for nanoparticle (NP) synthesis which has many advantages including its scalability, biocompatibility and applicability by utilizing water which acts as a reduction medium [7]. The vitamins, proteins, organic acids, amino acids, and secondary metabolites act as capping and stabilizing agents, and playing key role to reduce metal salts of synthesized NPs [8]. Green synthesis approach has more advantages over chemicals and physical ways of synthesizing NPs. Biological processes involve bacterial, fungal and plant enzymes that occupy complex procedures for sustaining cell cultures under aseptic conditions while significant production of NPs was achieved by employing plant extracts, encompassing simplicity and applicability with low energy consumption. However, chemically synthesized NPs comprise of toxic reagents that remain as residues along with particles and ultimately nurture toxicity problems within human system [9, 10].

Therefore, a biological approach using plant extract has drawn much interest because it is simple, safe and environmental friendly process. Formerly, an extensive research had been done on the utilization of different plants extracts such as leaf, inflorescence, root hair, seed, peel for the synthesis of AgNPs including aloe vera [11], *Mangifera indica* [9], Phoenix dactylifera [10], *Caesalpinia ferrea* [12] and Banana [13]. Onion has been reported to have numerous important properties such as antimicrobial, antioxidant, antiparasitic and anti-inflammatory activities [14]. Recently, it is found that onion can be used as a potential candidate for synthesis of AgNPs.

The aim of the present study was to evaluate the use of onion extract as a reducing agent for AgNPs formation to utilize it as a nanobiofertilizer. The synthesized AgNPs were then characterized by UV–vis spectroscopy, Scanning Electron Microscopy (SEM), and Fourier transform infrared (FTIR) spectroscopy. The synthesized onion nanobiofertilizer will be utilized as a foliar pray on tomato and brinjal to investigate its efficiency.

Materials and methods

Plant material

Onion used as a reducing agent for synthesis of AgNPs, was obtained from the local market. Tomato and Brinjal seeds were also procured form local market. Tomato and Brinjal were sown in plastic bags containing cocopeat and soil in 1:1 ratio. The each bag sown with 2 seed and after germination 1 plantlets was maintained in each bag.

Preparation of plant extracts

First of all onion peel was removed and washed with distilled water twice, then cut into small pieces. 10 g of onion weighted and blended with 100 ml distilled water using a high-speed mechanical blender. The obtained slurry was mixed with the 70% alcohol and stirred for 1 min to get homogenous slurry. The alcohol blended slurry was subjected to boiling with stirring for 4 min. After cooling the mixture was filtered by whatman paper 1 and stored at 40^0C for 24h. The precipitate was dissolved in distilled water and stored it for further use.

Preparation of silver nano-particles

Five ml of onion extract was mixed with 5, 10 and 20 ml of 1.0 mM $AgNO_3$ (analytical grade, Sigma Aldrich) with constant stirring at 60–65^0C for 5 min and incubated at room temp for 5 days. The AgNPs formation was observed by the color change as described by Lekshmi *et al.* [15]. The AgNPs were collected by centrifugation at 15,000g for 20 min. Dispersion of AgNPs in de-ionized water followed by centrifugation were repeated three times to confirm purification. Onion extract and AgNPs were stored as lyophilized powder.

Characterization of silver nanoparticles

The synthesized AgNPs where characterized by UV- visible spectroscopy, FTIR and SEM. The best concentration of AgNPs where chosen for further characterization based on the data obtained from UV-visible spectroscopy.

UV–vis spectroscopy

Synthesis of AgNPs was assured by measuring the UV–vis spectrum of the reaction mixture. The absorption spectrum was recorded over the range of 300–800 nm using UV–vis spectrophotometer.

Fourier transform infrared

FTIR measurements were used to identify the possible biomolecules associated with AgNPs formation. The FTIR spectra were recorded in the range of 400-4000 cm^{-1} by KBr pellet method (Shimadzu). 0.1 gm of potassium bromide was grinded to fine paste in mortar and pestle for 2 min and a small amount of liquid sample was mixed into it. The FTIR spectra was recorded by Shimadzu.

Scanning electron microscopy

The morphology of AgNPs was studied using SEM. Thin films of the sample were prepared on a carbon coated tape by just placing a very small amount of the sample on the grid, extra sample removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes. The SEM analysis was used to determine the structure of the reaction products that were formed.

Application of synthesized nanobiofertilizer in agriculture

Seeds of two vegetative crops (tomato and brinjal) were used and plants were cultivated in net house. The 15 days old plants were sprayed with the addition of different doses (5, 10 and 15 ml/l) of nanofertilizer extracted from Onion. The control plants were sprayed with water only. The spraying was done after every 2 days. After 7 days, data of plant vigor and biomass of plant was observed and recorded.

Results and discussion

In the present study, AgNPs synthesis by reducing Ag+ ions present in the aqueous solution of silver nitrate with the help of onion extract was investigated. The best reaction conditions were obtained with 5 ml onion extract and 10 ml 1.0 mM $AgNO_3$ (Fig. 1), the synthesis was confirmed by UV-Visible analysis. The color change from colorless to dark brown indicated the formation of AgNPs [16]. The change in the state of a matter from the molecular scale to nano scale is accompanied with color change due to excitation of surface plasmon vibrations (SPV) in NPs [17].

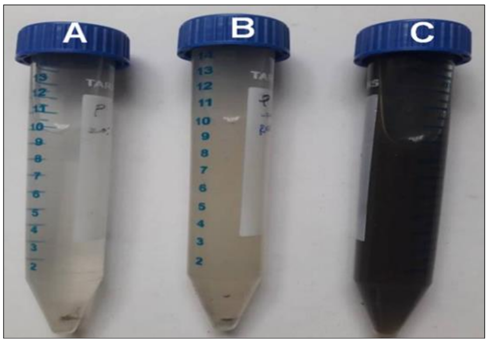

Fig 1: Synthesis of AgNPs of onion extract. A. 1.0 mM $AgNO_3$ solution, B. Onion extract, C. AgNO3 and Onion extract mixture showing color change after incubation of 5 days

Uv–vis spectroscopy

UV–vis spectroscopy is an important technique used to confirm the formation of metal NPs in an aqueous solution. The UV–vis absorption spectrum of the produced AgNPs showed an absorbance peak at 470 nm due to excitation of SPV in NPs. One of the most important features in the optical absorbance spectra of metal NPs is surface plasmon band, which is due to collective electron oscillation around the surface mode of the particles [18]. The UV-Vis spectroscopy analysis showed that the solution peak at an average wavelength of 437 nm Mohamed *et al*. [19]. Moreover, the UV-Vis spectral analysis cooper nanoparticles (CuNPs) showed peak at 565 nm is due to the surface SPR of Cu colloids formation of non-oxidized CuNPs by Marimuthu *et al*. [20].

FTIR analysis

The FTIR analysis was used to identify the possible bio-molecules responsible for capping and efficient stabilization of AgNPs synthesized by onion extract. The secondary metabolites are the main factors for the biosynthesis of AgNPs, the plant extract contain phenolic, alcohol, amine, carboxylic acid, alkaloids and terpenoids that responsible for reduction and stabilizing AgNPs [21]. As showed in Figure 3 some active functional groups in the synthesized AgNPs are confirmed by the presence of absorption bands in the range of 3679 to 400 cm^{-1}. The Figure 3 indicate the major peak at 3424 cm^{-1} and other peaks were obtained at 2921 cm^{-1}, 1625 cm^{-1}, 1387 cm^{-1}, 1061 cm^{-1} and 971 cm^{-1} (Fig. 3). The band at 3424 cm^{-1} corresponds to O–H stretching H-bonded alcohols and phenols. The peak at 2921 cm^{-1} and 1625 cm^{-1}corresponds to O–H stretch carboxylic acids and N–H bend primary amines respectively. The peak at 1387 cm^{-1} corresponds to C–N stretching of aromatic amine group. The bands observed at 1061 cm^{-1} and 971 cm^{-1} corresponds to C–N stretching alcohols, carboxylic acids, ethers and esters. The various study results were identified that alkynes, carbonyls, alkenes, alkyl halides, primary, secondary amines and aromatic groups are the natural products responsible for the reduction of Ag+ ions to AgNPs [22, 19]. The carbonyl group from the amino acid residues has the stronger ability to bind metal indicating that the proteins could prevent the molecules to be in clusters and stabilize AgNPs in the aqueous medium [23].

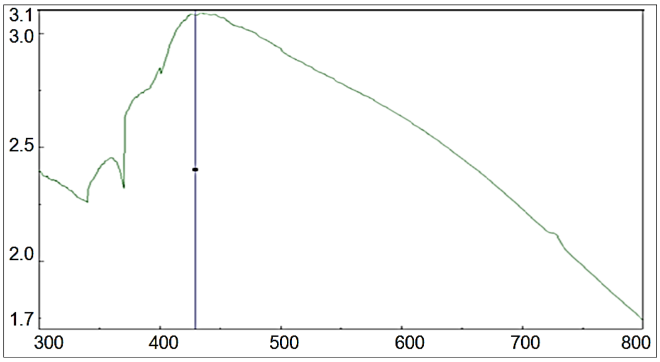

Fig 2: UV–vis absorption spectrum of the biosynthesized silver nanoparticles (AgNPs).

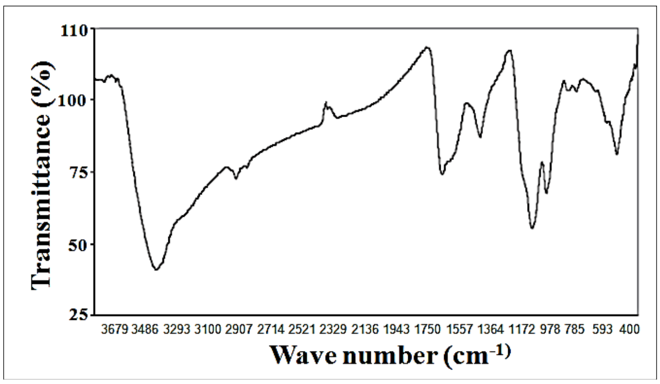

Fig 3: FTIR spectrum of the biosynthesized silver nanoparticles (AgNPs).

SEM analysis

The SEM technique is used to visualize the size and shape of NPs. The AgNPs were observed as cubical particles with the sizes between 10 to 30 nm, (Fig. 4). Similarly, various study used SEM to visualize the size and shape of NPs. The Solgi and Taghizadeh, [24] reported that SEM image show that SNPs are relatively uniform in diameter and have spherical shape. However, Kanagesan *et al*, [25] reported synthesis of nanorod shaped NPs produced with diameter range 10-30 nm.

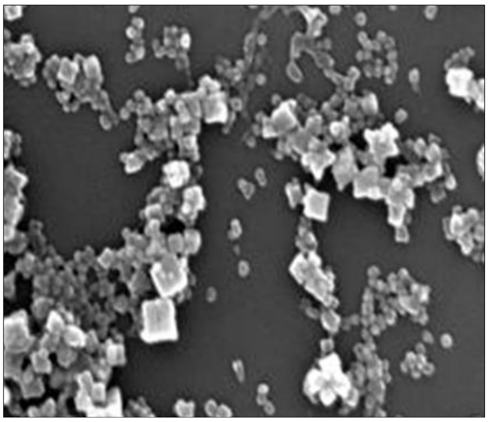

Fig 4: Scanning electron microscopy image of AgNps using onion extract

Application of synthesized nanobiofertilizer for crop improvement

The various concentrations of nanobiofertilizer such as 5, 10 and 15 ml/l were sprayed after every 2 days for up to 7 days. The vigor of the plants was observed and also the fresh weight of the harvested green plants was also measured. After the application of nanobiofertilizer, treated and non-treated plants were harvested and the fresh weights of the plants were taken. Both the fresh weight and vigor were higher in plants treated with 15 ml/l compared to 5, 10ml/l and control (Fig. 5). The increased fresh weight and vigor of nanobiofertilizer treated plant can be because of onion is rich in carbohydrates, proteins, sodium, potassium and phosphorus [1]. These mineral nutrients play a very important role in plant growth and development. Potassium citrate increases leaf area, improves leaf mineral content, enhances yield, and improves fruit quality [26]. Similarly, the application of nano silver liquid showed the highest dry and fresh weights compared to the control green onions [27].

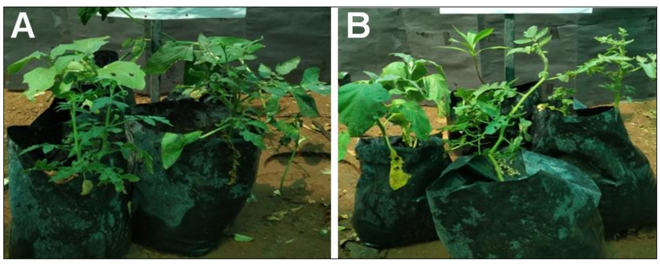

Fig 5: The effect of nanobiofertilizer treatment on tomato and brinjal. The 15ml/l of nanobiofertilizer sprayed for every 2 days up to 7days. A) treated plants of tomato and brinjal B) control plants are shown

Conclusion

We conclude that the nanobiofertilizer synthesized from onion extract is an effective fertilizer for brinjal, tomato plants and need to test it in field condition. The use of such types of nanobiofertiizer can reduce the environmental pollution and the excessive use of chemical fertilizers in the field and helps in reduction of farm management costs.

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Conflict of interest

The authors declare that they have no conflict of interest to publish this manuscript.

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