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# Sulfur Nanoparticles Improves Root and Shoot Growth of Tomato

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**Abstract**

The objective of this research work is to synthesize sulfur nanoparticles by green route and to investigate the beneficial effect on root and shoot growth of tomato. Sulfur nanoparticles (SNPs) synthesized using aqueous extract of *Ailanthus altissima* leaves at room temperature. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) inspections indicated that nanoparticles are spherical and polydispersed with diameters ranging between 5 and 80 nm. The potential of sulfur nanoparticles for enhancing tomato's growth, increasing the concentration of sulfur nanoparticles from 100 ppm to 300 ppm cause an increase in root and shoot lengths, while higher concentration 400 ppm and 600 ppm induced an inhibitory effect. Results of this study reveal that SNPs have the potential to enhance root and shoot growth of tomato and the effect is concentration dependent.

**Keywords:** green synthesis, sulfur nanoparticles, *A. Altissima*, tomato growth

## 1. Introduction

Sulfur has a wide range of applications in different industrial and apicultural activities. Sulfur nanoparticles have many advantages over micro-sulfur for their peculiar quantum size properties and high surface area. Sulfur acts as a soil conditioner in reducing the sodium content, and nitrogen fixing. For plants sulfur is necessary in the formation of proteins, amino acids, enzymes, vitamins, and chlorophyll. Also sulfur helps the plant's resistance to disease (Cooper et al., 1996; Dubuis et al., 2005). Recently, great effort has been focused on finding approaches to synthesis sulfur nanoparticles (SNPs) with well-defined shapes and nano-sizes. Among these, reverse microemulsion (Guo et al., 2006; Desphande et al., 2008), an electrochemical (Shamsipur et al., 2011), water-in-oil microemulsion (Soleimani et al., 2013), an aqueous solution of potassium polysulfide and various organic and inorganic acids (Massalimov et al., 2012), ultrasonic technique (Xie et al., 2009), green solvents (Xie et al., 2012), surfactant assisted route (Chauhuri & Paria, 2010), supersaturated solvent (Wu et al., 2008), chemical precipitation (Meenatchi & Renuga, 2015), and membrane as natural biomaterial (Cheng et al., 2011). These methods have many disadvantages due to the difficulty of scale up the process, separation and purification of nanoparticles from the micro emulsions and energy requirements. Several studies were conducted to investigate the role of metal nanoparticles on plants growth (Farghaly & Nafady, 2015; Salama, 2012; Hafeez et al., 2015; Du et al., 2011; Jayarambabu et al., 2014). In previous work (Salem et al., 2016) we have reported the effect of sulfur nanoparticles on the growth and development of *Cucurbita pepo*. As continuation of this work, sulfur nanoparticles synthesized by a green method using *Ailanthus altissima* leaves extract at room temperature and investigated the effect on tomato growth.

## 2. Materials and Methods

### *2.1 Materials*

Sodium thiosulfate pentahydrate, ($Na_2S_2O_3 \cdot 5H_2O$, 99.8%) and hydrochloric acid (HCl, 37%) were obtained from Merck, Darmstadt, Germany and used as received without any further purification. Sterile distilled water was used with conductivity 1 µS/cm.

### *2.2 Preparation of A. altissima Leaves Extract*

Fresh leaves of *A. altissima* were collected from the trees at campus of Royal Scientific Society, Jordan (Figure 1). The leaves were washed under running tap water to remove any dust attached to the leaves and subsequently with distilled water 3-4 times. The leaves were air dried for four weeks at room temperature and then finely powdered through grinding using Restch RM 100 grinder. The aqueous extract was prepared by mixing 40 g of powdered leaves with 500 mL of sterile distilled water and boiled for 10 min. The aqueous extract of leaves was separated by filtration with Whatman No.1 filter paper and then centrifuged at 1200 rpm for 5 minutes to remove heavy biomaterials. The filtrate was collected and stored at room temperature.

Figure 1. Photograph of fresh leaves of *A. altissima*

*2.3 Synthesis of Sulfur Nanoparticles*

Sulfur nanoparticles synthesized as follows: an appropriate amount of sodium thiosulfate pentahydrate $Na_2S_2O_3 \cdot 5H_2O$ was dissolved in 100 mL of *A. altissima* leaves extract under mild stirring for 5 minutes at room temperature. Afterwards hydrochloric acid (HCl, 16%) was added drop by drop under stirring for allowing the sulfur precipitations uniformly. The suspended sulfur particles obtained were separated by centrifugation at 1200 rpm/min for 5 minutes and then washed with distilled water several times to remove any biological materials. Sulfur nanoparticles after purification were dried in a vacuum at 60 °C for 6 h.

*2.4 Characterization Techniques*

Crystalline sulfur nanoparticles were characterized by X-ray diffractometer (XRD-600, Shimadzu, Japan), FTIR spectrophotometer (IR-Prestige-21, Shimaduz, Japan), and scanning electron microscopy-energy/dispersive X-ray spectroscopy (EDS) (Quanta FEI 450 SEM machine).

*2.5 Plant's Growth and Sulfur Nanoparticles*

Experiments were carried out under greenhouse. In all experiments, the indeterminately growing round tomato was used. After 10 days, plants were picked out and transferred to a field at the Royal Scientific Society, where they grow in soil of the field. The response of tomato plant to sulfur nanoparticles was studied through field experiments conducted during May-July of 2015. Sulfur nanoparticles (SNPs) were applied to the field soil 100 ppm–600 ppm doses.

*2.6 Root and Shoot Length*

Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transission zone up to the base of the cotyledons.

*2.7 Fresh and Dry Weight*

The fresh weight of root and shoot of seedlings was determined by weigh of the root and shoot separately on electric balance. After the fresh weight taken then the seedlings was kept in a hot air oven at 60 °C for 48 hrs then the weight of dry matter was recorded.

## 3. Results

*3.1 X-Ray Diffraction (XRD) Analysis*

The XRD analysis of the synthesized sulfur nanoparticles is illustrated in Figure 2. The 2θ peaks at 15.32°, 23.06°, 25.82°, 27.68°, 31.36°, 36.98°, 42.68°, 47.66°, 51.16° and the crystal planes of sulphur at 113, 222, 026, 040, 044, 317, 319, 515, and 266, respectively (Standard: JCPDS 08-0247). The average particles diameter of sulfur nanoparticles were determined by Debye-Scherrer formula (Klug & Alexander, 1974):

$$D = K \cdot \lambda / \beta \ \cos\theta \quad (1)$$

The unassigned peaks in XRD referred to crystalline phases of *A. altissima* leaves extract. The crystalline size of sulfur nanoparticles calculated from Scherrer equation was about 20 nm.

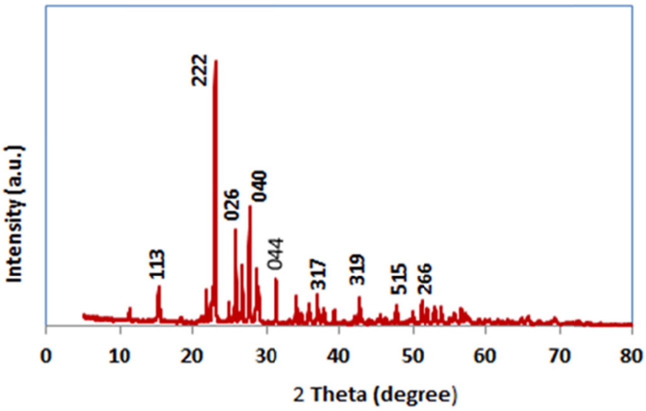


Figure 2. XRD pattern of synthesized sulfur nanoparticles

### *3.2 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis*

FT-IR spectrum of *A. altissima* extract, Figure 3 display strong broad absorption bands at 3444 $cm^{-1}$ could be ascribed to the stretching absorption band of amino (-NH) and hydroxyl (-OH) stretching H-bonded alcohols and phenols. The absorption peaks at 2920 $cm^{-1}$could be assigned to the asymmetric and symmetric stretching of -CH, $-CH_2$ and $-CH_3$ functional groups of aliphatic. The peak at 1735 $cm^{-1}$ corresponds to stretching to carboxyl group. The bands at 1616 $cm^{-1}$ is characteristic of amide carbonyl group in amide I and amide II. The bands at 1543 $cm^{-1}$ and 1450 $cm^{-1}$ are assigned to the methylene scissoring vibrations from the proteins. C-N stretch of aromatic amines and carboxylic acids gives rise to band at 1327 $cm^{-1}$. The band at 1215 $cm^{-1}$ is due to C-O vibrations of alcohols, phenols and C-N stretching vibrations of amine. The band at 1041 $cm^{-1}$ assigned to the C-O stretching vibrations of alcohols. The peaks at 879 $cm^{-1}$, 775 $cm^{-1}$, and 605 $cm^{-1}$ can be assigned to aromatic compounds.

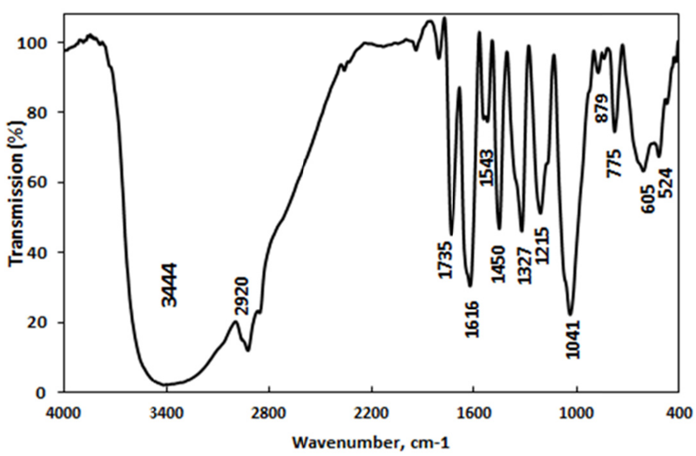


Figure 3. FT-IR spectrum of *A. altissima* leaves extract

FT-IR spectra of the synthesized SNPs indicate a new chemistry linkage on the surface of sulfur nanoparticles. This suggests that *A. altissima* leaves extract can bind to sulfur nanoparticles through carbonyl of the amino acid residues in the protein of the extracts, therefore acting as stabilizer and dispersing agent for synthesized sulfur nanoparticles and prevent agglomeration of sulfur nanoparticles. The FT-IR spectrum of the sulfur nanoparticles, Figure 4 shows a strong and sharp peak at 466 $cm^{-1}$. The strong peaks at 3410 $cm^{-1}$, 2920 $cm^{-1}$, 1647 $cm^{-1}$ indicated that the biomaterials of plant leaves act as capping agent and also to stabilize the nanoparticles.

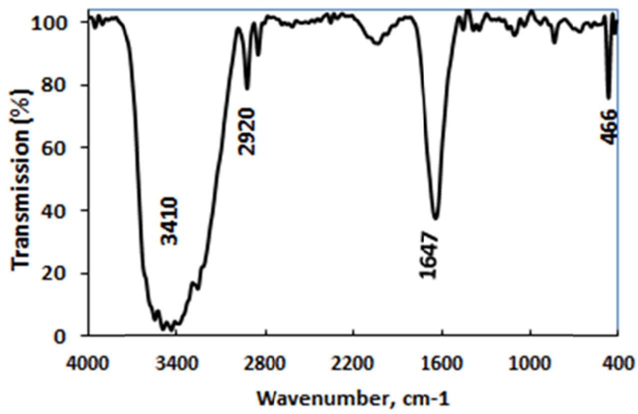


Figure 4. FT-IR spectrum of synthesized sulfur nanoparticles

### *3.3 Scanning Electron Microscopy (SEM)-Energy-Dispersive X-Ray Spectroscopy (EDS) Analysis*

SEM analysis of the synthesized sulfur nanoparticles shows that all the particles are homogeneously dispersed and with average diameter size about 40 nm and spherical in shape with few exceptional as ellipsoidal. Mineral concentration analysis to root and shoot of tomato using energy-dispersive X-ray spectroscopy analysis, Figure 5 indicated that absorption of sulfur nanoparticles by tomato roots was about 2.04 wt. %. However, shoots absorption to sulfur nanoparticles, Figure 6 was about 4.14 wt. %. These results suggest that sulfur nanoparticles absorbed by interacting with biomaterials of the plant forming organosulfur compounds, which help the plant to grow more healthy, greener, and with stronger root system grow more healthy, greener, and with stronger root system.

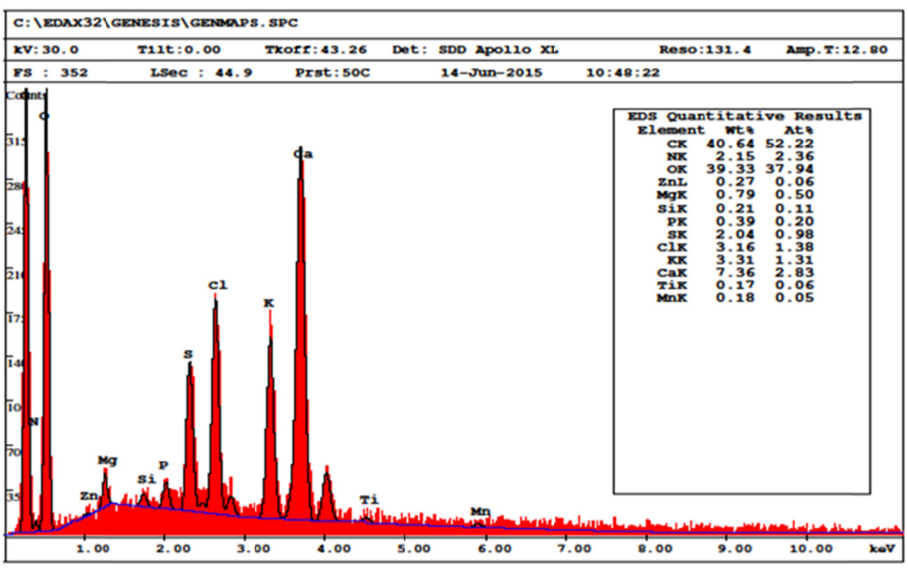


Figure 5. Energy-dispersive X-ray spectroscopy (EDS) analysis for metal concentrations in tomato root

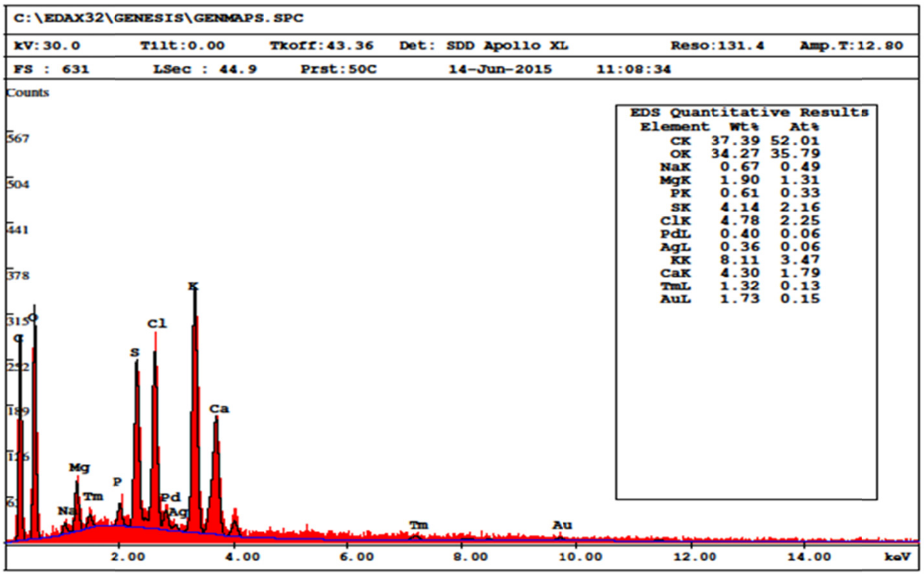


Figure 6. Energy-dispersive X-ray Spectroscopy (EDS) analyses for metal concentrations in tomato shoot

### *3.4 Tomato's Growth*

Effect of sulfur nanoparticles on root and shoot growth of tomato is presented in Table 1. Analysis of data indicated that different concentrations of SNPs are significantly influence the root and shoot lengths. At higher concentrations of SNPs > 400 ppm caused a drop in values of the growth of root and shoot lengths. Tomato plants treated soil with 300 ppm SNPs were visibly compact and greener in colour and produce healthier plant with stronger root system, Figure 7. The reduction in root and shoot growth at higher doses > 400 ppm may be attributed to toxic level of nanoparticles. The results showed that the tomato root and shoot measurements of as expressed by root fresh weight, dry weight of root, shoot fresh weight, and dry weight of shoot were influenced by different concentrations of SNPs. The dry root and shoot weight were significantly different among the SNPs treatments.

Table 1. Effect of sulfur nanoparticles (S-NPs) on growth parameters of tomato

| Treatments | Concentration of sulfur nanoparticles (SNPs) on growth of tomato | | | | | |
|---|---|---|---|---|---|---|
| | Control | 100 ppm | 200 ppm | 300 ppm | 400 ppm | 600 ppm |
| Root length (cm) | 8.2e | 9.8d | 14.4b | 18.6a | 13.6b | 11.4c |
| Shoot length (cm) | 38.8e | 43.6d | 54.3b | 68.9a | 50.7c | 48.4c |
| Fresh weight of root (g) | 3.5e | 4.2d | 5.6c | 7.7a | 5.9b | 5.3c |
| Dry weight of root (g) | 0.42e | 0.53c | 0.66b | 0.98a | 0.46d | 0.41e |
| Fresh weight of shoot (g) | 22.2c | 22.6c | 26.4b | 29.7a | 18.8d | 16.2e |
| Dry weight of shoot (g) | 5.1c | 5.4b | 5.6b | 6.2a | 5.9c | 5.3b |

*Note*. Values with different letters are significantly different at $P < 0.05$.

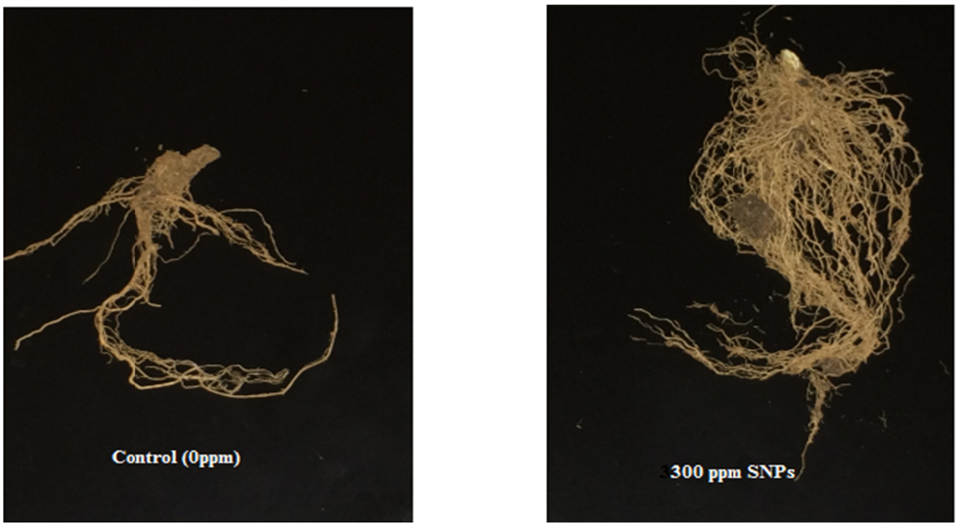


Figure 7. Photograph shows the root of control tomato and the one treated with 300ppm SNPs

## 4. Discussion

In current study the synthesized SNPs by green route using *A. altissima* leaves extract were characterized by different techniques for calculation of crystalline size, particles size, morphology, and chemical compositions. The averge particle size diameter was ranging between 5-80 nm. The suspended sulfur nanoparticles formed by this route indicated that *A. altissima* leaves extract act as capping agent and stabilized the SNPs. The different concentrations of SNPs nanoparticles effect on tomato growth and the length of root and shoot were studied. Nanoparticles of sulfur are quickly transported through the plant and included in the metabolic processes through forming organsulfur compounds, which are necessary to plant growth. We observed in tomato growth, 300 ppm concentration of SNPs suspension solution shown good shoot, root growth results compared with other concentrations and control in this experiment**.** The present study demonstrated the effect of green synthesized sulfur nanoparticles on tomato growth. The maximum effect of SNPs on root length, shoot lengths, fresh and dry weights of root and shoot of tomato was found at 300 ppm. Beyond this concentration > 400 ppm, the roots and shoots growth, fresh and dry weights of roots and shoots were declined. The effective growth at certain SNPs concentration may be attributed to the absorption of SNPs by roots and shoots and formed organosulfur compounds, which help in enhancing the growth as necessary gradient for plant growth. Further, higher concentrations of SNPs > 400 ppm caused a drop in values of the growth of root and shoot lengths. Tomato plants planted in soil treated with 300 ppm SNPs were visibly compact, vigorous, and greener in color with stronger root system.

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