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Seed Germination and Growth of Cucumber (*Cucumis sativus*): Effect of Nano-Crystalline Sulfur

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Abstract

The present paper is focused on green synthesis of high purity sulfur nanoparticles (SNPs) and its effect on seed germination and seedling growth of cucumber (*Cucumis sativus*). Synthesized SNPs were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (SEM-EDS). The crystalline size of synthesized SNPs as calculated by Scherer equation was 40 nm. SEM analysis of the SNPs is in spherical shape and with a diameter size between 5-80 nm. In the present study, different concentrations of SNPs were used for the treatment of cucumber seeds to study the effect on bioavailability of seed germination and seedling growth of cucumber. The results of this experiment showed that an increase in concentrations of SNPs had significantly increased seed germination and seedling growth of cucumber.

Keywords: sulfur nanoparticles, cucumber, seed germination, bioavailability, rosemary leaves extract

1. Introduction

Nanoparticles have distinctly different size diameter, surface area, chemical and biological activities compared to both individual molecules and bulk materials with the same chemical composition. Sulfur is an essential element for plants. It works as nitrogen-fixing nodules on legumes, in the formation of chlorophyll, proteins, amino acids, vitamins, and enzymes, the plant's resistance to diseases. In fact soils get sulfur from airborne particles, the weathering of minerals in soil, and decomposition of organic materials by microbial activity. Different nanomaterials were used to study their effect on seed germination and seedling growth such as titanium oxide TiO_2 (Silva et al., 2016; Nithiya et al., 2015; Gao et al., 2013; Samadi et al., 2014), zinc oxide ZnO_2 nanoparticles (Lin & Xing, 2007; Jayarambabu et al., 2014; Raskar & Laware, 2014), copper oxide CuO nanoparticles (Moon et al., 2014), iron oxide Fe_2O_3 nanoparticles (Kumar et al., 2015; Canivet et al., 2015), silver nanoparticles (Parveen & Rio, 2015; Razzaq et al., 2016; Hojjat, 2015), nano-crystalline powders of Fe, Co, and Cu (Ngo et al., 2014), Nano-SiO_2 (Siddiqui & Al-Whaibi, 2014), and aluminum oxide nanoparticles (Juhel et al., 2011), However, some of these nanomaterials have many disadvantages due to the difficulty of scale up the process of synthesis, and toxic materials. Developing facile and green methods for synthesizing sulfur nanoparticles are of importance and still a challenge for materials researchers.

The importance of sulfur nanoparticles in different applications, such as antimicrobial agents, fertilizers, and insecticides, the development of green methods is highly essential for Nano-sized sulfur particles. In previous work, we studied the effect of sulfur nanoparticles on plant's growth (Salem et al., 2016). As continuation of our previous work, the main objective of this study is to investigate the effect of green synthesized sulfur nanoparticles by rosemary (*Rosmarinus officinalis*) leaves aqueous extract on seed germination and seedling growth of cucumber.

2. Materials and Methods

2.1 Materials

Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, 99.5%), hydrochloric acid (37%, HCl), and ethanol (99.8%) were obtained from E Merck, Darmstadt, Germany. Fresh rosemary (*Rosmarinus officinalis*) leaves were obtained from in and out of the Royal Scientific Society, Jordan. Double distilled and deionized water was utilized for the preparation of leaves extract.

2.2 Preparation of Rosemary (Rosmarinus officinalis) Leaves Aqueous Extract

20 g of dried powder of *rosemary* leaves were mixed with 500 mL deionized water and heated at 80 °C for 10 min. Afterwards the mixture was then cooled at room temperature. The aqueous extract was obtained by filtration on filter paper Whatman No. 1 to remove solid particles. Then the extract centrifuged at 1200 rpm for 5 min to remove heavy biomaterials. The filtrate was stored at room temperature for further experimental work.

2.3 Synthesis of Sulfur Nanoparticles (SNPs)

In this experiment 1.2 g of sodium thiosulfate pentahydrate was dissolved in 100 ml of *rosemary* leaves aqueous extract under stirring on magnetic stirrer at room temperature. Afterwards 10% hydrochloric acid was added drop wise to the sodium thiosulfate solution under stirring for allowing the sulfur precipitations uniformly. The suspended sulfur particles obtained were then centrifuged at 1000 rpm for 5 min at ambient temperature. The supernatant was discarded and the precipitate was repeatedly washed with distilled water and absolute ethanol to get rid any biological materials. The product was finally dried in a vacuum at 60 °C for 4 h for characterization.

2.4 Seeds

The cucumber seeds were purchased from National Seeds, Jordan and prior to starting the experiments; cucumber seeds were stored in dry conditions in the dark to avoid any potential loss of their viability.

2.5 Seeds Germination and Exposure

The seeds were checked for their bioavailability by suspending them in deionized water. The seeds settled to the bottom were selected for further study. The seeds were immersed in a 5% dimethyl sulfoxide (DMSO, $C_2H_6SO_2$, E Merck, Germany) solution 10 min for sterilization and consistency of all experiments. Afterwards cucumber seeds were rinsed three times in deionized water and then soaked in a SNPs suspension at concentrations 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 600 ppm, for 4 h in an incubator at 27 °C. Healthy and uniformly sized seeds were selected and then snow at equal distance in prepared soil pots. Seed germination experiments were carried out with 6 sets. First set considered as control (0 ppm SNPs) for comparison with the treated ones. Each treatment was carried out with three replicates and the results were presented as a mean standard deviation (±SD).

2.6 Seed Germination Application

The seed germination percentage (S_g%), was calculated from the following formula (Jayarambabu et al., 2014):

$$S_g\% = \frac{S_c}{S_s} \times 100 \tag{1}$$

Where, S_s is the number of seed germinated in sample and S_c is the number seed germinated in control.

2.7 Fresh and Dry Mass

The fresh and dry mass of root and stem was quantified through weighing in precision scale. The dry mass of root and shoot was determined after placed in an oven at 60 °C for 24 h giving constant weight.

2.8 Statistical Analysis

Each treatment was conducted with three replicates and the results were presented in mean standard deviation (±SD). All treatments were compared to those controls using t-test paired two samples for means determined at a 5% confidence level ($p < 0.05$).

3. Results and Discussion

The XRD pattern of green synthesized sulfur nanoparticles by rosemary leaves aqueous extract is illustrated in Figure 1. The 2θ peaks at 15.26°, 21.68°, 22.86°, 25.64°, 27.52°, 31.21°, 33.44°, 36.84°, 42.54°, 47.52°, and 51.04° are attributed to the crystal planes of sulfur at (113), (131), (222), (040), (313), (044), (400), (422), (319), (515), and (226), respectively. The sulfur nanoparticles are well-crystalline and the position and the relative intensity of the diffraction peaks match well with the standard monoclinic phase sulfur diffraction pattern (JCPDS N-34-094). The average particle size of the synthesized sulfur nanoparticles was about 20 nm as calculated using Debye-Scherrer formula (Klug & Alexander, 1954).

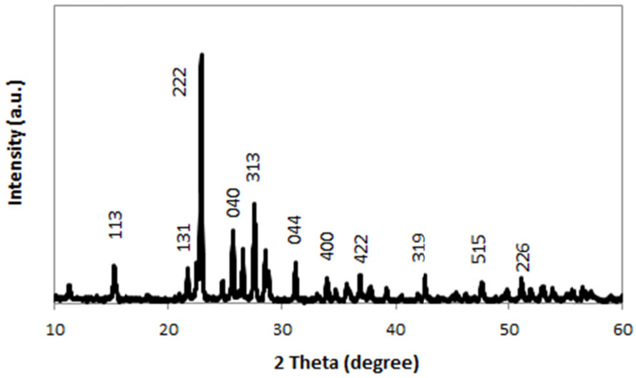

Figure 1. XRD pattern of synthesized sulfur nanoparticles using *Rosemary leaves* aqueous extract

FT-IR spectra of *rosemary* leaves aqueous extract is illustrated in Figure 2. A strong and abroad absorption bands at 3424 cm^{-1} could be ascribed to the stretching absorption band of amino (-NH), hydroxyl (-OH) stretching H-bonded alcohols and phenols. The absorption peaks at 2916 cm^{-1} and 2846 cm^{-1} could be assigned to the asymmetric and symmetric stretching of $-CH_2$ and $-CH_3$ functional groups of aliphatic. The shoulder peak at 1701 cm^{-1} corresponds to stretching carboxyl groups. The band at 1620 cm^{-1} is characteristic of amide carbonyl group in amide I and amide II. The band 1415 cm^{-1} is assigned to the methylene scissoring vibrations from the proteins. C-N stretch of aromatic amines and carboxylic acids gives rise to band at 1373 cm^{-1}. The band at 1022 cm^{-1} assigned to the C-O stretching vibrations of alcohols. The broad peak at 523 cm^{-1} can be assigned to aromatic compounds. These functional groups act as dispersing, capping and stabilizing agents for SNPs during the process of synthesis.

FT-IR spectra of the synthesized SNPs, Figure 3 indicated a new chemistry linkage on the surface of sulfur nanoparticles. This suggests that *rosemary* 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 prevent agglomeration of sulfur nanoparticles. The main characteristic peaks of rosemary leaves extract were observed in FT-IR spectra of sulphur nanoparticles. The FT-IR spectrum of the sulfur nanoparticles shows a strong and sharp peak at 462 cm^{-1}.

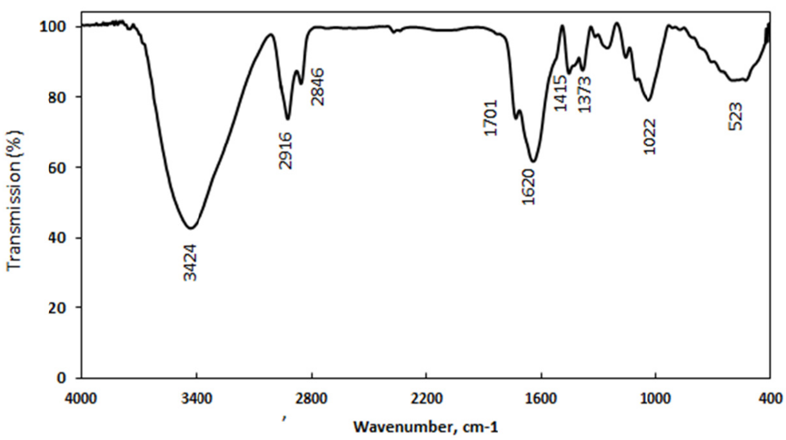

Figure 2. Fourier infrared spectrum of *Rosemary* leaves extract

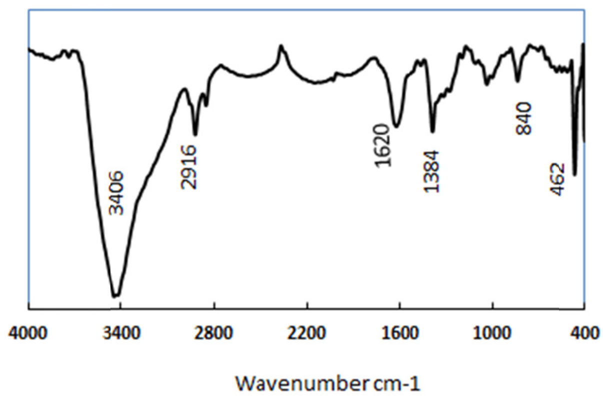

Figure 3. Fourier infrared spectrum of synthesized sulfur nanoparticles

Scanning electron microscopy (SEM) images of synthesized sulfur nanoparticles are illustrated in Figure 4. The crystals of sulfur nanoparticles are spherical in shape. The average diameter particles size is approximately in the range of 20-80 nm.

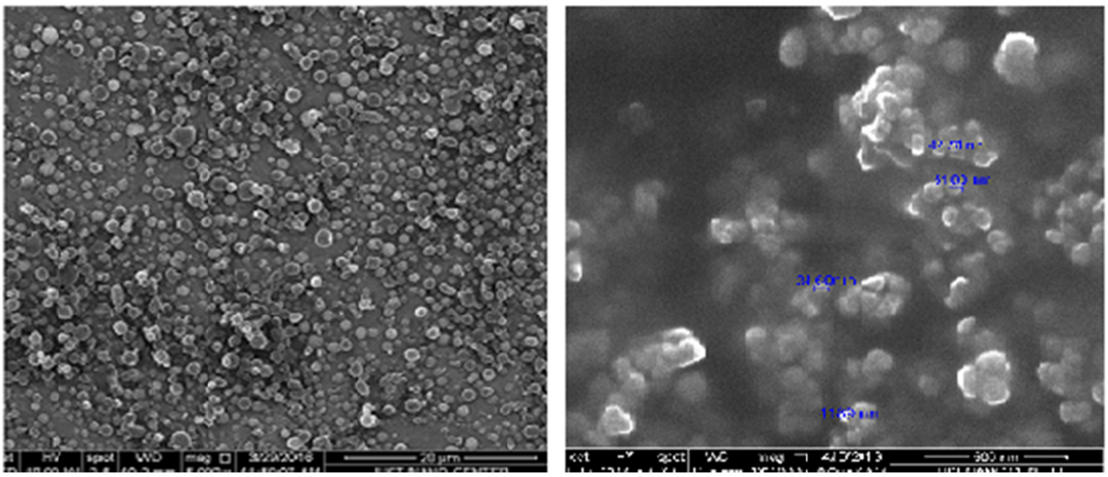

Figure 4. SEM images of synthesized SNPs using *rosemary* leaves aqueous extract

Percentage of seed germination was significantly affected by the interaction of SNPs. The results the control has shown the 75% germination. Cucumber seeds treated with SNPs have shown an increase in germination at different concentrations, viz., 100 ppm shows 90%, 200 ppm-600 ppm show a 100% in germination, Table 1. Control showed statistically significant difference and could not improve root and stem lengths.

Table 1. Growth charactestics of cucumber at different SNPs concentrations within 12 days

SNPs (ppm)	SG (%)	Root length (cm)	Stem length (cm)
Control	75	4	10
100	90	5	10.6
200	100	5.6	10.9
300	100	6.1	11.6
400	100	8.2	13.4
600	100	8.4	13.5

In the present study, the sulfur nanoparticles showed an increase in root and stem lengths with increasing the concentration of SNPs. At low concentrations 100 ppm of SNPs showed less effect on root and stem. The control showed 75% germination. The increase in root and stem growth at higher doses may attributed to the importance of sulfur in building chlorophyll, proteins, amino acids, vitamins. Also SNPS helps the plant's resistance to diseases and helping the plant's growth.

The stem fresh and dry weight was found to be influenced by different concentrations of SNPs, Table 2. Figures 5 and 6 showed the effect of different concentrations of SNPs on cucumber root growth and increasing number of seminal roots. Sulfur nanoparticles can stimulate cucumber's growth.

Table 2. Effect of sulfur nanoparticles (SNPs) on seed germination of cucumber

Treatment with SNPs (ppm)	Root fresh Wt. (g)	Root dry Wt. (g)	Stem fresh Wt. (g)	Stem dry Wt. (g)
Control	0.006	0.0034	0.33	0.0123
100	0.012	0.0054	0.43	0.0169
200	0.016	0.0057	0.49	0.0172
300	0.019	0.0060	0.53	0.0180
400	0.024	0.0068	0.59	0.021
600	0.026	0.0069	0.62	0.23

Figure 5. Photos show the positive effect of SNPs on root growth compared with control

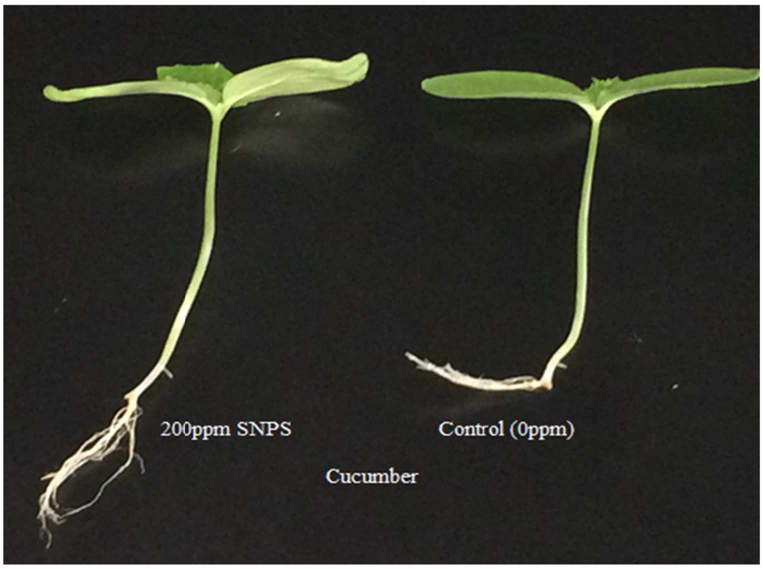

Figure 6. A photographs for cucumber plant showed the difference in root growth, the one seeds treated with 200 ppm SNPs and the control 0 ppm

4. Conclusion

In this research paper the synthesized SNPs by rosemary leaves extract were characterized by different techniques for determining the crystalline size, particles size, and morphology. Different concentrations (100-600 ppm) of synthesized sulfur nanoparticles effect on cucumber seed germination and the root and shoot lengths. These results indicated that sulfur nanoparticles are necessary in the process of formation of proteins, amino acids, enzymes, chlorophyll, and resistance to disease, therefore aids in cucumber growth.

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