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Green synthesis of sulfur nanoparticles using *Punica granatum* peels and the effects on the growth of tomato by foliar spray applications

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ABSTRACT

Sulfur nanoparticles (SNPs) have been successfully prepared from sodium thiosulfate in the presence of *Punica granatum* peels aqueous extract at room temperature. The resulting sulfur nanoparticles were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) equipped with energy-dispersive X-ray spectroscopy (EDS). Highly crystalline synthesized sulfur nanoparticles exhibiting high purity, spherical shape with average particle size of about 50 nm applied at a rate of 100 ppm, 200 ppm, and 300 ppm as foliar spray for tomato leaves. The obtained results revealed that the foliar spraying tomato leaves with 200 ppm sulfur nanoparticles are very beneficial to plant growth and produced healthy plant with greener leaves and high quality of tomato fruits compared with control.

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1. Introduction

Sulfur is widely used as a fungi toxic agent in agricultural fields against a wide range of powdery mildews, certain smut and rust fungi and some other common fungal pathogens including *Cladosporium fulvum*, and *Fusarium* spp. Sulfur is enzymatically reduced in living cells to form hydrogen sulfide and this reduction proceeds by a single electron transfer from the donor in the cell to sulfur (Owens, 1963). Production of excess hydrogen sulfide within the fungal body spells enhanced toxicity and leads to the inhibition of fungal growth. Despite the antifungal efficacy of sulfur, its use is now restricted among farmers and agrochemical industries mainly for two reasons, firstly sulfur is required in bulk quantities for application in agricultural fields and secondly it is also likely to induce resistance in the target species. It is desirable to considerably reduce the amount, so that the cost is cut down and at the same time, the buildup of resistance in target pathogens is minimized. Intelligent bottom up nanoscience application is the most appropriate tool to resolve the aforesaid problems. Sulfur nanoparticles can be synthesized by different methods such as water-in-oil microemulsion technique (Guo et al., 2006), liquid phase chemical precipitation (Guo et al., 2005), from H_2S gas, using novel biodegradable iron chelates in W/O microemulsion (Deshpande et al., 2008), and aqueous surfactant solutions (Chaudhuri and Paria, 2010). Among these methods, application of green synthesis technique is the simplest of all. The process can be carried out easily by using *Punica granatum* (pomegranate) peels at room temperature and with minimal facility. The effects of various nanomaterials on plants growth were studied. These nanomaterials include cerium (IV) oxide, CeO_2 and copper oxide, CuO (Hong et al., 2015), cerium dioxide and zinc oxide nanoparticles (Peralta-Videa et al., 2014), nano titanium oxide, TiO_2 (Gao et al., 2013), nano zinc oxide, ZnO, nano iron oxide, FeO and nano-ZnCuFe-oxide particles (Dhoke et al., 2013), iron oxide and titanium oxide nanoparticles (Burke et al., 2015), cobalt ferrite, $CoFe_2O_4$ (López-Moreno et al., 2016), silver nanoparticles, Ag (Larue et al., 2014), iron nanoparticles, Fe (Canivet et al., 2015), alumina, Al_2O_3 (Juhel et al., 2011), and magnesium nanoparticles, Mg (Indira and Tarafdar, 2015). These nanomaterials used to study their effects on plant growth contain toxic heavy metals such as Zn(II), Co(II), and Ag(I) ions to soil, plant, and humans, Sulfur is considered as safer element due to its ability to form organo-sulfur compounds in plant tissue, which are necessary to grow plants healthy and antifungal activity (Hawkesford, 2000; Kim et al., 2006; Bakry et al., 2015; Suleiman et al., 2015). As continuation of our previous work on sulfur nanoparticles synthesis and its effect on plant growth (Salem et al., 2016a,b), a new green and large-scale method using *Punica garantum* peels for synthesis sulfur nanoparticles (SNPs).

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The main objectives of this study is to synthesize sulfur nanoparticles by green method using *Punica granatum* peels extract and to investigate the impact of a foliar exposure to SNPs on tomato plant leaves.

2. Experimental

2.1. Materials

Sodium thiosulfate pentahydrate ($Na_2S_2O_3{\cdot}H_2O$, 99.5%), hydrochloric acid (32%, HCl) were obtained from Merck, Darmstadt, Germany. Fresh *Punica granatum* (Pomegranate) peels was obtained from local Jordan market. Double distilled and deionized water was utilized for the preparation of aqueous extracts.

2.2. Preparation of aqueous extract of Punica granatum *peels*

20 g of dried powder of *Punica granatum* peels were mixed with 500 ml distilled water and boiled for 20 min. The mixture was then cooled at room temperature, followed 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)

Sulfur nanoparticles were synthesized as follows: 24.8 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3{\cdot}5H_2O$) was dissolved in 500 ml of *Punica granatum* peel extract under mild stirring for 10 min at room temperature and then diluted with 500 ml deionized water. Afterwards 10% HCl was added drop by drop under stirring for allowing the sulfur precipitations uniformly. The suspended sulfur particles obtained were then centrifuged at 5000 rpm for 10 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. In *Punica granatum* peels extract and acidic solution, sodium thiosulfate ($Na_2S_2O_3$) undergoes through a disproportionation reaction to sulfur and sulfonic acid according to:

$$Na_2S_2O_3{\cdot}5H_2O + 2HCl \rightarrow 2NaCl + S\downarrow + SO_2 + 6H_2O$$

2.4. Foliar applications of SNPs

Tomato (*Solanum lycopersicum*) plants were cultivated in a greenhouse where the temperature was maintained at $26 \pm 4\,^{\circ}C$ during day light and $20 \pm 2\,^{\circ}C$ during night. Experiments were con-

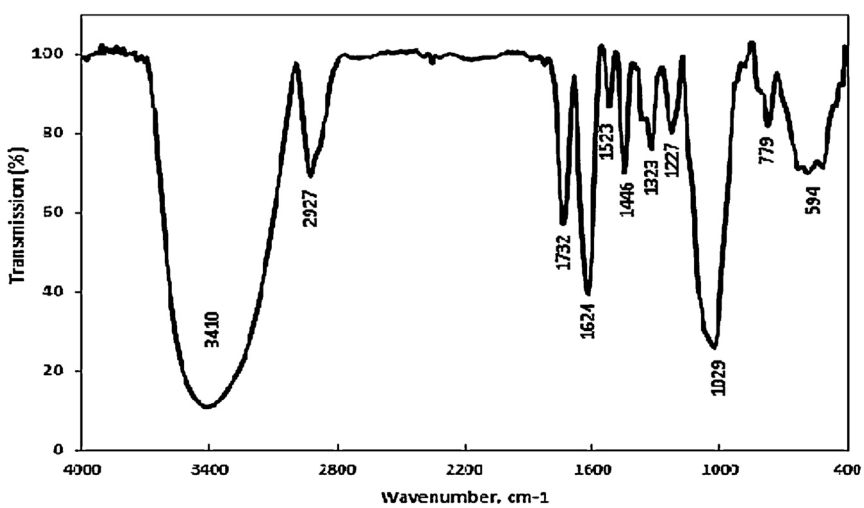

Fig. 1. FT-IR of *Punica granatum* peels aqueous extract.

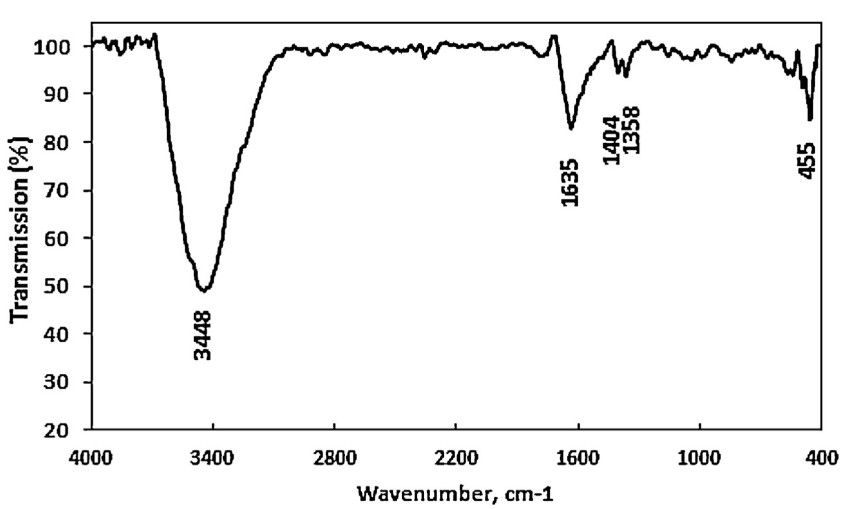

Fig. 2. FT-IR of the synthesized sulfur nanoparticles.

ducted in greenhouse, which was part of the Department of Plant Protection, Faculty of Agriculture, the University of Jordan. After 14 days sowing in trays filled with a commercial potting soil, plants were picked out and transferred to a field at the Royal Scientific Society, where grown in soil of the field. The response of tomato to sulfur nanoparticles was studied through field experiments conducted during May–June 2015. When tomato plants were three weeks old in the field, the leaves of the tomato plant were sprayed with 100 ppm, 200 ppm, and 300 ppm synthesized sulfur nanoparticles by green method. Aqueous solution of sulfur nanoparticles were sprayed with a hand-sprayer plastic bottle. After 7 days, foliar spray with SNPS was repeated. The soil was protected by with plastic sheets to prevent contamination with SNPs. Three replicates for each experiment were determined. Two weeks after treatment one plant from each group was harvested to determine sulfur nanoparticles SNPs and mineral contents in tissues of tomato plant. Control tomato plants were not treated with SNPS in order to be compared with that treated with SNPs.

2.5. Analysis of tomato plant

For sulfur nanoparticles analysis tomato plants were harvested after 24 days, washed with tape water several times and then with distilled water. The leaves and fruits of tomato samples were dried in an oven at 60 °C for 24 h. The concentrations of sulfur nanoparticles were determined by scanning electron microscopy (SEM) equipped with enemy-dispersive X-ray spectroscopy (EDS)

2.6. Statistical analysis

Each experiment was conducted with three replicates and the results were presented as mean ± SD (Standard deviation). The data recorded on various parameters were subjected to the analysis of variance (ANOVA) technique to find out the difference between different cultivars. In cases where differences were found significant, means were compared for differences using least significant difference (LSD) test. Statistical computer Software was applied for computing both the ANOVA and LSD. All the assumptions were checked to ensure the statistical validity of analysis.

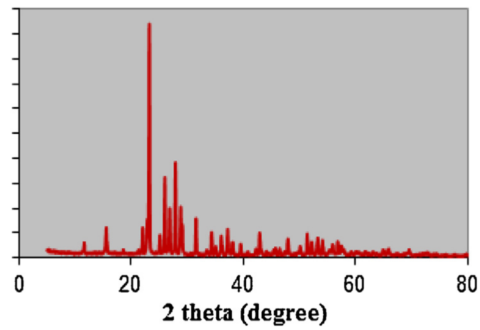

Fig. 3. XRD analysis of synthesized sulfur nanoparticles.

3. Results and discussion

3.1. FT-IR analysis of *P. granatum* peels aqueous extract

FT-IR spectra of *P. granatum* peels aqueous extract, Fig. 1 display strong and abroad absorption bands at 3410 cm^{-1}, which could be ascribed to the stretching absorption band of amino (-NH) and hydroxyl (—OH) stretching H-bonded alcohols and phenols. The strong absorption peak at 2927 cm^{-1} could be assigned to the asymmetric and symmetric stretching of $—CH_2$ and $—CH_3$ functional groups of aliphatic. Peak at 1732 cm^{-1} corresponds to stretching carboxyl groups. The band at 1624 cm^{-1} is characteristic of amide carbonyl group in amide I and amide II. The bands at 1523 cm^{-1} and 1446 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 1323 cm^{-1}. The band at 1227 cm^{-1} is due to C—O vibrations of alcohols, phenols and C—N stretching vibrations of amine. The band at 1029 cm^{-1} assigned to the C—O stretching vibrations of alcohols. The peaks at 779 cm^{-1}, and 594 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 indicate a new chemistry linkage on the surface of sulfur nanoparticles. This suggests that *P. granatum* peels extract can bind to sulfur nanoparticles

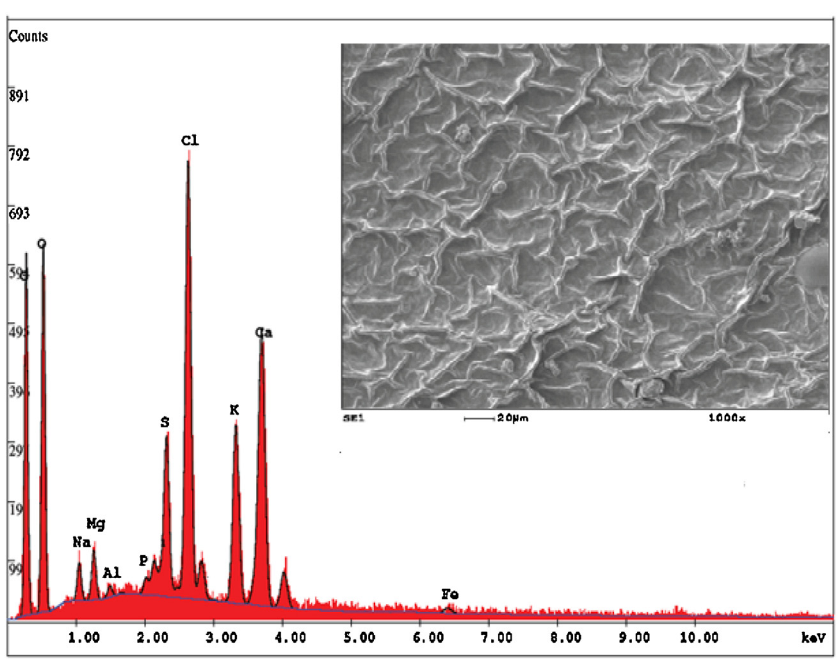

Fig. 4. SEM and EDS analysis of tomato leaves.

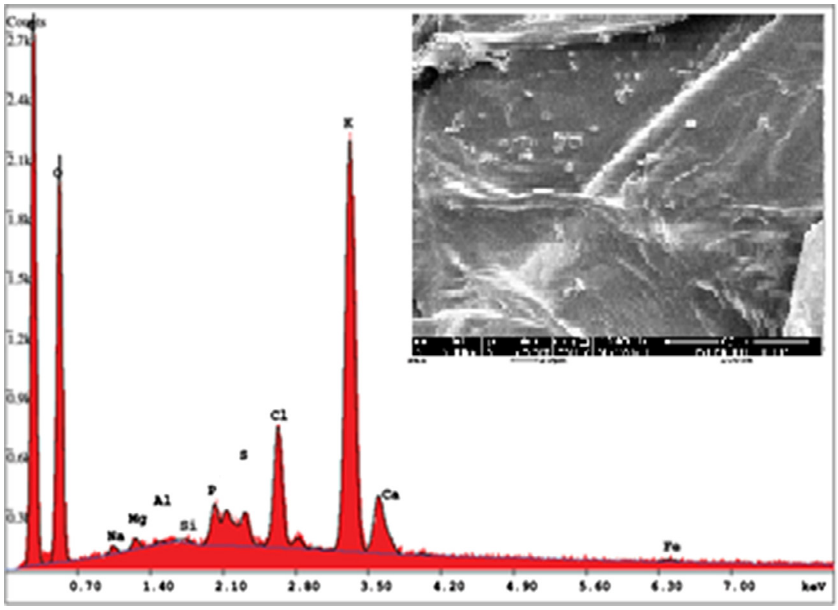

Fig. 5. SEM and EDS analysis of tomato lfruits.

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 main characteristic peaks of *Punica granatum* peels extract were observed in FT-IR spectra of sulphur nanoparticles, Fig. 2. The FT-IR spectra of the sulfur nanoparticles show a strong and sharp peak at 455 cm^{-1}.

3.2. XRD analysis of synthesized sulfur nanoparticles

The powder X-ray diffraction (XRD) pattern, Fig. 3 indicated the presence of sulfur nanoparticles using *Punica granatum* peels aqueous extract. 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 (Joint Committee on Powder Diffraction Standards, JCPDS No-34-0941). The average particle size of the synthesized sulfur nanoparticles calculated using Debye-Scherrer formula (Cullity, 1978) was found to be 20 nm.

3.3. Scanning electron microscopy

Scanning electron micrographs of the synthesized sulfur nanoparticles using aqueous extract of *Punica granatum* peels contains particles with spherical shapes and in the size diameter range of 10–40 nm. SEM images depicts crystals of various shapes, leaves inclusion in leaves, and sheets crystals in shape in fruits

3.4. Effect of sulfur nanoparticles on growth parameters

An example of SEM-EDS analysis of tomato leaves and fruits is shown in Figs. 4 and 5. The shape of SEM images depicts crystals of various shapes, leaves inclusion in leaves, and spherical crystals in shape in fruits.

Data in Table 1 shows the effects of foliar treatment with SNPs on tomato leaves. The results illustrated that tomato plant treated with 200 ppm SNPs recorded significant increases of SNPs absorption compared with control plants. Greener and healthier leaves of tomato were observed in the plants treated with 200 ppm SNPS. These observations is a result of interaction of SNPs absorbed by leaves with organic compounds of tomato tissues forming organic-

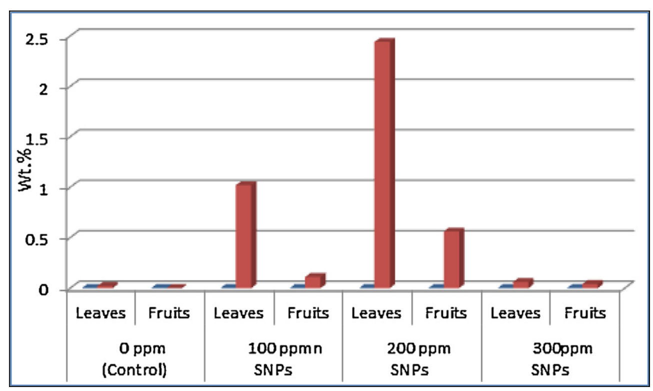

Fig. 6. Average of three replicates weight percent (%) of SNPs content in leaves and fruits.

sulfur compounds, which help in building the chlorophyll and nitrogen content of the leaves. In addition part of these organic compounds under atmosphere decompose and release hydrogen sulfide H_2S, which acts as antimicrobial agent.

Fig. 6 shows that the SNPs, at 200 ppm significantly increased weight percent of sulfur content in leaves and fruits. In this study, also it was observed that tomato leaves sprayed with 200 ppm SNPs are greener and healthier than those of control leaves.

Table 2 shows the effects of foliar spray of sulfur nanoparticles on leaves of tomato. The plant height and root increase with increasing sulfur nanoparticles up to 200 ppm and then decrease with 300 ppm. This indicates that the organic materials in plant tissue needs proper quantity of SNPs to form organic-sulfur compounds, which helps the plant to grow healthy and to defend it self and has antimicrobial activity.

4. Conclusion

Highly crystalline sulfur nanoparticles exhibited high purity, spherical shape with average particle size of about 20 nm, and particle size distribution in range 10pnm to 40pnm were synthesized using *P. granatum* peels aqueous extract. The obtained results raveled that the foliar spraying tomato leaves plant with sulfur nanoparticles are very beneficial to plant growth. Foliar sprayed

Table 1
Effects of sulfur nanoparticles (SNPs) on leaves and fruits of Tomato plant.

	0 ppm (Control)		100 ppm SNPs		200 ppm SNPs		300 ppm SNPs	
Element	Leaves (Wt.%)	Fruits (Wt.%)	Leaves (Wt.%)	Fruits (Wt.%)	Leaves (Wt.%)	Fruits (Wt.%)	Leaves (Wt.%)	Fruits (Wt.%)
C	46.2 ± 0.8	51.3 ± 0.27	46.6 ± 0.35	51.5 ± 0.46	41.7 ± 0.38	46.1 ± 0.27	42.3 ± 0.31	44.6 ± 0.51
O	36.1± 0.2	43.5 ± 0.26	36.1 ± 0.21	43.5 ± 0.27	33.6 ± 0.78	38.2 ± 0.55	31.8 ± 1.04	35.9 ± 0.45
Na	0.2± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.03	1.4 ± 0.41	0.3 ± 0.07	0.2 ± 0.03	0.2 ± 0.03
K	7.9 ± 0.25	2.2 ± 0.21	7.9 ± 0.25	2.2 ± 0.20	0.1 ± 0.03	9.1 ± 0.45	1.2 ± 0.02	1.0 ± 0.03
Mg	0.7± 0.3	0.2 ± 0.02	0.7 ± 0.31	0.21 ± 0.02	1.2 ± 0.30	0.3 ± 0.08	0.6 ± 0.03	0.1 ± 0.02
Ca	2.0 ± 0.21	0.3 ± 0.02	2.0 ± 0.22	0.31 ± 0.026	6.2 ± 0.35	0.4 ± 0.03	3.4 ± 0.4	0.2 ± 0.03
Fe	BDL	0.3 ± 0.06	BDL	0.3 ± 0.036	0.3 ± 0.05	0.2 ± 0.04	0.1 ± 0.03	0.1 ± 0.02
Si	0.2 ± 0.02	0.3 ± 0.06	0.2 ± 0.02	0.3 ± 0.04	BDL	BDL	BDL	BDL
Al	0.1 ± 0.02	1.3 ± 0.08	0.1 ± 0.04	1.4 ± 0.36	0.2 ± 0.03	0.1 ± 0.01	0.3 ± 0.05	0.1 ± 0.03
P	0.7 ± 0.05	0.1 ± 0.02	0.7 ± 0.27	0.11 ± 0.02	0.3 ± 0.05	0.7 ± 0.04	0.81 ± 0.06	BDL
Cl	3.1 ± 0.17	0.5 ± 0.02	3.3 ± 0.18	0.51 ± 0.03	7.2 ± 0.04	2.1 ± 0.03	2.7 ± 0.25	0.2 ± 0.01
S	BDL	BDL	1.0 ± 0.02	0.11 ± 0.03	2.5 ± 0.31	0.60 ± 0.03	0.1 ± 0.01	BDL

BDL = Below Detection Limit.

Table 2
Effect of foliar Sulfur nanoparticles (SNPs) spray on tomato growth.

SNPS ppm	Plant hight (cm)	Fresh weight (g)	Root length (cm)	Root fresh weight (g)
0	60.4 ± 0.35	2.65 ± 0.05	12.1 ± 0.43	0.45 ± 0.03
100	68.5 ± 0.15	3.42 ± 0.04	13.5 ± 0.22	0.52 ± 0.04
200	98.5 ± 0.35	4.89 ± 0.09	16.4 ± 0.40	0.78 ± 0.05
300	70.6 ± 0.47	2.88 ± 0.12	11.6 ± 0.41	0.48 ± 0.03
LSD 0.05	0.94	0.44	0.42	0.11

The mean difference is significant with P < 0.05.

with sulfur nanoparticles at 200 ppm (200 mg/L) produce healthy and high quality of tomato fruits compared with control. However, a detailed studies and experiments are carried out in order to give complete picture and understanding the effects of sulfur nanoparticles on tomato and cucumber plants. Foliar sprayed with SNPs was found acting on increasing the potassium content in fruits of tomato compared with control.

Conflict of interest

The authors declare that they have no competing interests

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