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Green synthesis of sulfur nanoparticles using *Rosmarinus officinalis* leaves extract and nematicidal activity against *Meloidogyne javanica*

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ABSTRACT

Sulfur nanoparticles (S-NPs) were prepared using rosemary (*Rosmarinus officinalis*) leaves aqueous extract and sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) at room temperature. The S-NPs were characterized by UV-visible spectrophotometer, Fourier transform infrared (FT-IR), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and X-ray diffraction (XRD) techniques. The sulfur nanoparticles are crystalline in nature with average size of 40 nm. The morphology of S-NPs could be controlled by tuning the amount of rosemary leaves aqueous extract and sulfur ions. The cytotoxic effects of the S-NPs on hatching of second stage root-knot and mortality of second stage of root-knot nematode (*Meloidogyne javanica*) were tested. These results confirmed that the S-NPs synthesized using rosemary leaves aqueous extract and could possibly be used as nematicidal to manage *M. javanica* infestation.

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Capsule Summary: Biogenic synthesis of sulfur nanoparticles using *Rosmarinus officinalis* leaves aqueous extract was reported and prepared S-NPs showed promising nematicidal activity against *Meloidogyne javanica*.

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INTRODUCTION

Root-knot nematode (R-KN) is the most economically important plant parasitic nematode species that cause serious damage to most agricultural crops. More than 3000 plant species are hosts to this pest. The use of nematicides is considered the main management technique to decrease these nematode effects. However, nematicides do not provide long term suppression of R-KN. Not only that but also there are restriction of nematicide because of environmental and human health concerns. Alternatives are needed to be one of the integrated nematodes management. Nanoparticles, as

alternative, have a potential to suppress growth of some fungi, they showed fungicidal effect while they are not toxic to human cells (Huang et al., 2019; Ibrahim et al., 2019; Montiel-Rozas et al., 2019; Sharma et al., 2018).

Different methods to synthesize sulfur nanoparticles (S-NPs) with well-defined shapes and nano-sizes were reported using aqueous surfactant (Chaudhuri and Paria, 2010), eggshell membrane (Cheng et al., 2011), W/O microemulsion (Guo et al., 2006; Desphande et al., 2008; Soleimani et al., 2013), precipitation method using sodium thiosulphate and tetraoctylammonium bromide surfactants in hydrochloric acid media (Suleiman et al., 2015; Meenatchi and Renuga, 2015) and electrochemical method (Shamsipur

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. Developing facile and green methods for synthesizing sulfur nanoparticles are of importance and still a challenge for materials researchers (Alvarez-Bayona et al., 2019; da Silva et al., 2019; Jayarambabu et al., 2020; Pan et al., 2019; Ravichandran et al., 2019; Vidovix et al., 2019). Using plants extracts as reducing, stabilizing agents for green synthesis of S-NPs were reported in the literature such as *Melia Azedarach* leaves extract (Salem et al., 2016a), *Ailanthus altissima* leaves extract (Salem et al., 2016b), *Punica granatum* leaf extract (Salem et al., 2016c), herbal surfactant (Kouzegaran and Farhadi, 2017), *Albizia julibrissin* fruits extract (Awwad et al., 2015) and *Syzygium aromaticum* bud extract (Rajesh et al., 2018).

Based on aforementioned facts, present study was designed to synthesize sulfur nanoparticles via green route using rosemary leaves aqueous extract at room temperature (27°C), which were characterized by advanced techniques and nematicidal activity was evaluated against *M. javanica*

MATERIAL AND METHODS

Preparation of rosemary leaves aqueous extract

Fresh healthy leaves of rosemary were obtained from a nearby field at Royal Scientific Society, Amman, Jordan. Rosemary leaves were cleaned with running tap water followed by deionized water to remove impurities. Leaves were dried at our laboratory (27°C) for one week. Dried Rosemary leaves were pulverized in a blender to obtain powder with uniform size. Rosemary powder (10 g) was added to a 500 ml glass beaker with 400 ml de-ionized water and boiled for 10 minutes and cooled at room temperature. Afterwards, a filtration process was carried out through Whatman filter paper No. 1. Filtrate was kept at 4 °C for use in synthesis of sulfur nanoparticles at room temperature.

Synthesis of sulfur nanoparticles (S-NPs)

Sodium thiosulfate pentahydrate (1.2 g) ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) was dissolved in 100 mL of rosemary leaves aqueous extract under stirring 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 of any biological material. The product was finally dried in a vacuum at 60 °C for 4 h.

Characterization of synthesized S-NPs

The synthesized S-NPs were characterized by different techniques such as Fourier transform infrared spectroscopy (FT-IR, Shimadzu, IR-Prestige-21), UV-vis spectrum (Schimadzu 1601 spectrophotometer) of nanoparticles was recorded, by taking 0.1 ml of the sample and diluting it with 2 ml deionized water, as a function of time of reaction in the wave length region 200–700 nm operated at a resolution of 1 nm. The crystalline structure of the nanoparticles were characterized by X-ray powder diffraction (XRD, Shimadzu, XRD-6000 with $\text{CuK}\alpha$ radiation $\lambda = 1.5405 \text{ \AA}$ over a wide range of Bragg angles ($30 \leq 2\theta \leq 80$). The morphology and size distribution of synthesized S-NPs nanoparticles was characterized using scanning electron microscope (SEM, a Hitachi S-4500 SEM machine) and transmission electron microscope (TEM-JEOL 1010) operated at an accelerating voltage of 100 kV.

Nematicidal activity evaluation

Nematode culture: Populations of the root-knot nematode (RKN) *Meloidogyne javanica* were isolated from infected cucumber plants grown in the Jordan Valley. Temporary and permanent mounts of females processed and species were identified using original descriptions and diagnostic keys.

Effect of S-NPs on hatching of nematode: Three egg masses of the tested species of R-KN species were handpicked from galled cucumber roots and were placed in each well of a 6 wells tissue culture plate that contained a total of 5 ml of either 30 and 60 ppm of S-NPs. Egg masses were placed in water only or were treated with the plant leaf extract (rosemary, 30 and 60 ppm) served as controls. The plates were incubated for one week at $\pm 25 \text{ }^\circ\text{C}$. The hatched J₂s were counted after seven days using a dissecting microscope. After one week of exposure, treated egg masses with Sulfur nanoparticles were transferred to petri dishes containing fresh water and incubated for five days to monitor J₂s hatching. This test was performed to test if the effect of sulfur nanoparticles was static or cidal. Each treatment was replicated three times in a completely randomized design. Results were tabulated and analyzed.

Effect of S-NPs on mortality of nematode: Egg masses were handpicked from cucumber roots using a dissecting microscope and then were placed in plastic petri dish (3.5 cm in diameter) containing fresh water and incubated at $\pm 25 \text{ }^\circ\text{C}$ until the second stage juveniles (J₂s) hatched. A total of 50 hatched J₂s were placed in each well of a 6 wells tissue culture plate that contained a total of 5 ml of either 30 and 60 ppm of S-NPs. Second stage juveniles placed in water only or treated with the plant extract (rosemary, 30 and 60 ppm) served as controls. Petri dishes of all treatments were incubated for four days at $\pm 27^\circ\text{C}$. Each treatment was replicated five times in a completely randomized design. Dead J₂s were counted after 4 days of exposure using a dissecting microscope and then the mortality percentage was calculated. Results were tabulated and analyzed.

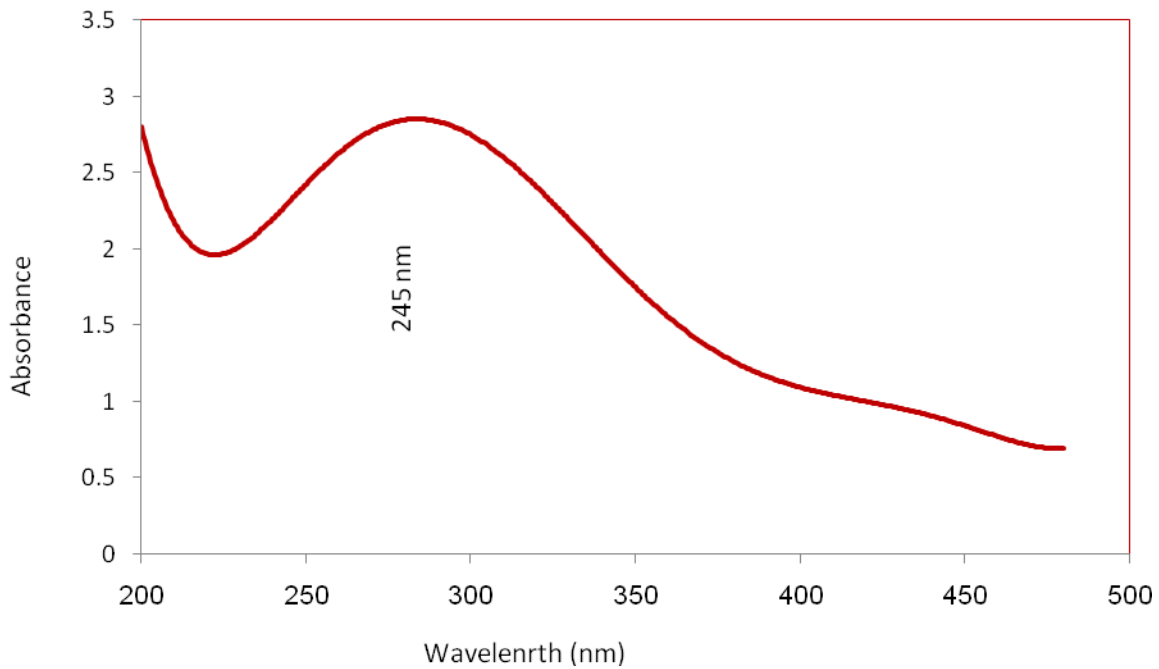


Fig. 1: UV-vis spectrum of sulfur nanoparticles (S-NPs) synthesized using rosemary leaves aqueous extract

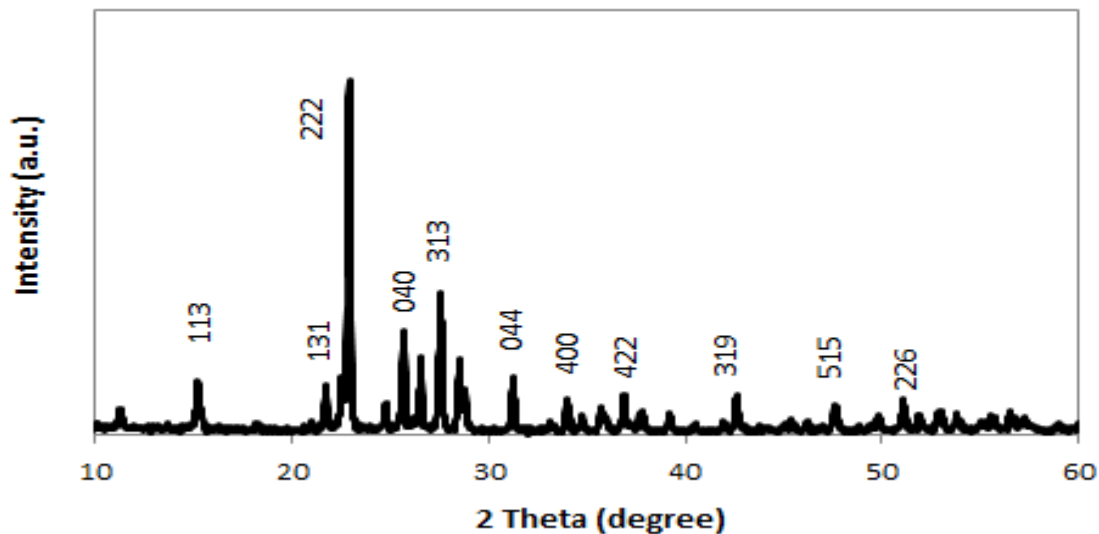


Fig. 2: XRD pattern of synthesized sulfur nanoparticles using Rosemary leaves aqueous extract

Statistical analysis

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

RESULTS AND DISCUSSION

UV-vis spectroscopy study

The mixture of rosemary leaves extract and sulfur nanoparticles solution was subjected to UV-vis spectra, based on the color change and the absorbance of the reaction medium was noted. Fig. 1 showed surface

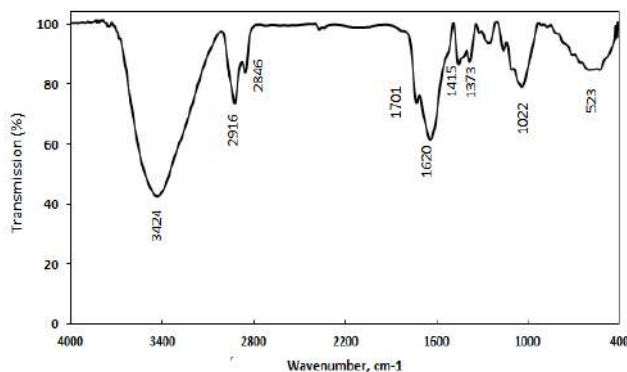


Fig. 3: FT-IR analysis of rosemary leaves aqueous extract

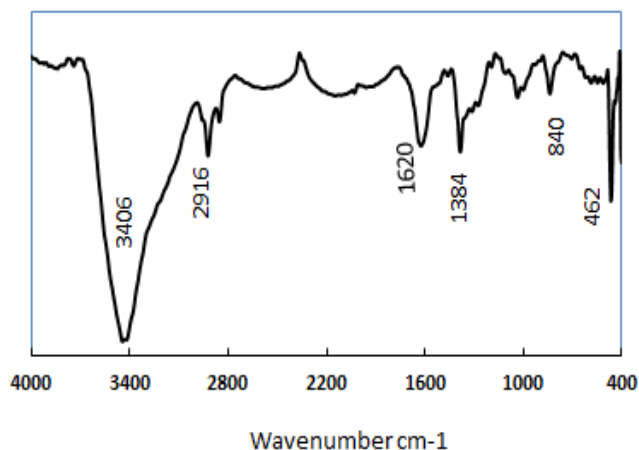


Fig. 4: FT-IR analysis of synthesized sulfur nanoparticles (Y-axis = Transmittance, %)

Plasmon resonance (SPR) bands of the colloidal sulfur nanoparticles were centered at around 245 nm. In the present study, the absorption spectra of sulfur nanoparticles synthesized using rosemary leaves extract reveals the conversion of sulfur ions to sulfur nanoparticles with almost 100%, as evidenced by qualitative testing of supernatant after the purification of sulfur nanoparticles.

X-ray diffraction (XRD) analysis

The XRD pattern of green synthesized sulfur nanoparticles by rosemary leaves aqueous extract is illustrated in Fig. 2. 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 and Alexander, 1954).

Fourier transforms infrared (FT-IR) analysis

FT-IR spectrum of rosemary leaves aqueous extract is illustrated in Fig. 3. A strong and broad 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 $-\text{CH}_2$ and $-\text{CH}_3$ 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 S-NPs during the process of synthesis. FT-IR spectra of the synthesized S-NPs, Fig. 4 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} .

Scanning and transmission electron microscopy (SEM and TEM) analysis

Scanning and transmission electron microscopy images of the synthesized sulfur nanoparticles are illustrated in Fig. 5. The crystals of sulfur nanoparticles are spherical in shape. The average diameter of particle size is in the range of 5-80 nm.

Nematicidal activity

Effect of sulfur nanoparticles on hatching of second stage root-knot nematode (Meloidogyne javanica): Results showed that treatment of egg masses of *M. javanica* with S-NPs at the two concentrations (30 and 60 ppm) caused a total inhibition of J2s hatching after seven days of exposure and were significantly differed from those treated with plant extract and water only. The inhibition of hatching continued after placing the treated egg masses in water and this confirms that the effect of the S-NPs is tidal and not static. On the other hand, a total of 113 and 181 J2s of *M. javanica* hatched from egg masses exposed to 30 and 60 ppm of the plant extract for seven days (Fig. 6).

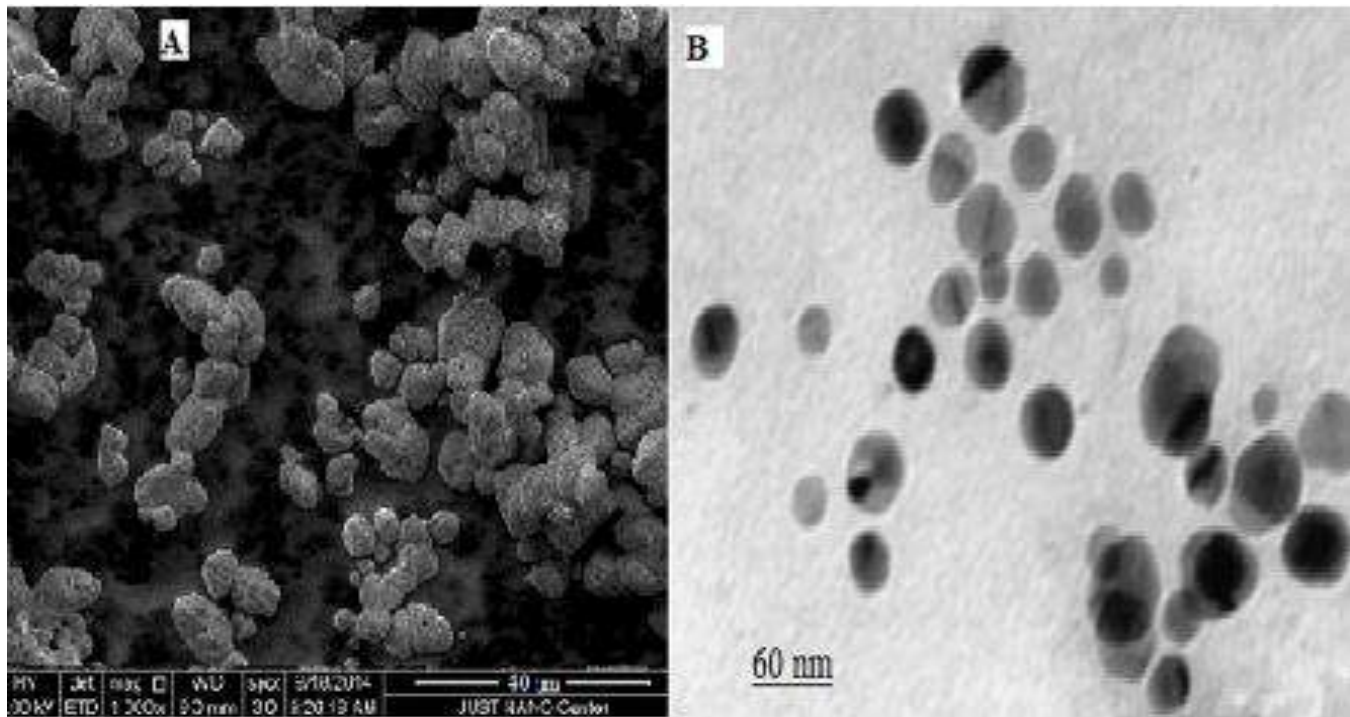


Fig. 5: SEM (A) and TEM (B) images of synthesized sulfur nanoparticles

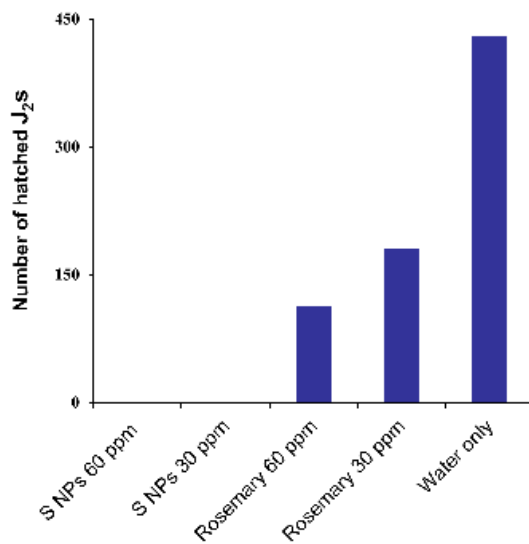


Fig. 6: Effect of sulfur nanoparticles on hatching of J₂s from egg masses of *M. javanica*

Effect of sulfur nanoparticles on mortality of second stage of root-knot nematode (Meloidogyne javanica): The J₂s of *M. javanica* were highly sensitive to the two concentrations of the S-NPs since 100% mortality of J₂s occurred after 4 days of exposure. Results showed that the plant extract, Rosemary had a few nematicidal effect on the treated J₂s but significantly was higher than those placed in water alone Fig. 7. Findings revealed that S-NPs have potential to

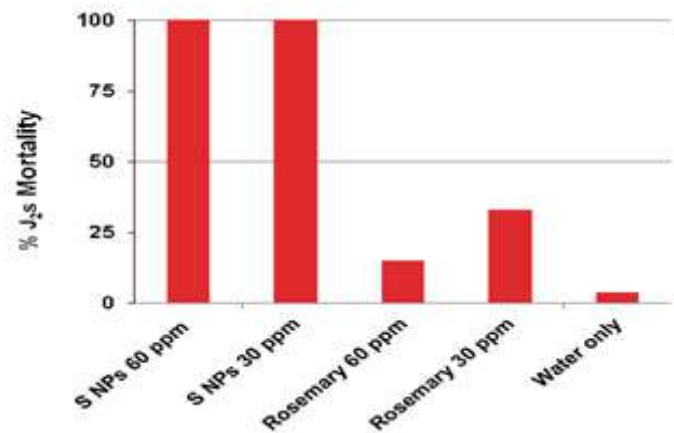


Fig. 7: Effect of sulfur nanoparticles on mortality of J₂s of *M. javanica* at different exposure times

control the *Meloidogyne javanica* infestation and also these findings are in line with previous studies that NPs showed promising nematicidal activity (Dong et al., 2018; Kalaiselvi et al., 2019; Liang et al., 2018; Marimon-Bolívar et al., 2019; Yang et al., 2017). The green synthesis of NPs for biological applications proved to be highly efficient since it is eco-benign technique (Hamedi and Shojaosadati, 2019; Hunagund et al., 2017; Muniyappan and Nagarajan, 2014; Patra et al., 2016; Rajiv et al., 2017; Thangamani and Bhuvaneshwari, 2019).

CONCLUSIONS

A facile green route for synthesis sulfur nanoparticles from sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in the presence of rosemary leaves aqueous extract was developed. The particle size of sulfur nanoparticles (S-NPs) could be controlled from 5nm to 80nm. The synthesized nanoparticles were characterized by XRD, FT-IR, SEM and TEM techniques. The obtained results showed that the green synthesized sulfur nanoparticles have spherical shape with average size diameter 40 nm. In view of high efficiency, the sulfur nanoparticles are expected to have applications in agricultural field to control nematodes infestation.

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