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Research Article

Green synthesized silver nanoparticles using Malva *Malva sylvestris* as a potential management strategy for root-knot nematode *Meloidogyne javanica* on tomato *Solanum lycopersicum*

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Abstract: Root-knot nematodes, *Meloidogyne* spp. cause 5% of the global crop losses. Silver nanoparticles (AgNPs) are a potentially effective nematicide. There are many methods to synthesize metallic nanoparticles, including using organic compounds, which are clean and environmentally friendly. In the present studies, the efficacy of as-synthesized AgNPs using *Malva sylvestris* extract (Ms-AgNPs) on egg hatching and mortality of the second-stage juveniles (J2) of *M. javanica in vitro*, and on the growth parameters of nematode infected tomato plant under greenhouse conditions was investigated. The results showed that increasing Ms-AgNPs concentration decreased the hatching rate and increased the mortality of J2. Ms-AgNPs at 107, 164 and 250 ppm reduced J2 numbers by 30, 50, and 70%, respectively, and were established as lethal doses for nematode control. Ms-AgNPs at a dosage of 250 ppm reduced the number of eggs in the root system and the nematode reproduction factor by 60.4 and 56.3%, respectively, and increased shoot length and shoot fresh weight by 11.2 and 14%, respectively. Ms-AgNPs at a very low concentration reduced the population of *M. javanica* without causing any harmful effects on tomato. Therefore it can be used in nematode control strategies due to its environmentally friendly composition.

Keywords: Control, microwave-assisted extraction, plant-mediated synthesis

Introduction

Root-knot nematodes *Meloidogyne* spp. damage tomato crops by up to 50% (Natarajan *et al*., 2006). Methods of controlling plant-parasitic nematodes include agronomic measures, cultivation of resistant cultivars, biocontrol, use of trap plants, intermittent cultivation, sanitation, sunbathing, and chemical nematicides. Despite the variety of nematode control methods, none is effective for controlling this pathogen due to the different limitations of each technique mentioned (Abdellatif *et al*., 2016; Nassar, 2016). For example, biocontrol agents have disadvantages such as the limited range of their effect and the uncertainty of their application in the field due to the different environmental conditions (Crow *et al*., 2011). Therefore, it is essential to choose

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more effective and environmentally friendly strategies to control plant parasitic nematodes (Fernandez *et al*., 2001). Nanometer-sized materials are increasingly produced and used because they have exciting properties (Rajeshkumar *et al*., 2013; Sadeghi *et al*., 2015). Silver nanoparticles have recently been proposed to control plant pathogens, including nematodes (Roh *et al*., 2009; Lara *et al*., 2011). Because of the multitude of antimicrobial mechanisms of nanoparticles, microorganisms cannot adapt to or develop resistance to silver (Sadeghi *et al*., 2014). Unfortunately, most processes leading to the production of nanoparticles inevitably involve the use of hazardous substances and pose severe threats to the environment (Senapati *et al*., 2012). Some nanoparticle manufacturing processes use toxic solvents that inevitably produce unnecessary and dangerous substances, often requiring high energy consumption (Osaka *et al*., 2006; Peng *et al*., 2006). Therefore, researchers turned to bio-friendly methods or green chemistry to find a clean, non-toxic, harmless solution (Khan *et al*., 2017). There is a long list of resources used for the bio-production of metallic nanoparticles, including yeast and plants (Singh *et al*., 2015). Green-synthesized silver nanoparticles (AgNPs) have received much attention due to their low cost and environmental compatibility (Mittal *et al*., 2013).

So far, the biological production of silver nanoparticles has been carried out using plants such as *Piper longum* L. (Jacob *et al*., 2012), *Achras sapota* L. (Thakore *et al*., 2019), and many other plants with biocidal properties (Rajabi *et al*., 2017; Moradi-Alvand *et al*., 2019a, 2019b; Mirsadeghi *et al*., 2019). The static and nematicidal properties of the plant extract due to secondary metabolites have been demonstrated in many studies (Singh *et al*., 2017; Rafiee *et al*., 2019). Mallow (*Malva sylvestris* L.) grows as a common weed in many places and is cultivated for medicinal purposes (Lust, 2014). The main components of *M. sylvestris* are phenols and flavonoid compounds, and the presence of malvin, delphinidin, malvidin, β-carotene, cyanin, tannin, saponins, alkaloids, mucilage, and especially antioxidants, which were detected in vitro (Al-Rubaye *et al*., 2017; Hasimi *et al*., 2017). *M. sylvestris* showed high mortality rates for nematode eggs and J2s (Alikarami *et al*., 2017).

Due to the environmental hazards of using chemical pesticides and the tendency to find suitable substitutes for these compounds, several studies have been conducted to find compounds with nematicidal properties, including organic compounds of plant origin. Considering the effective role of *M. sylvestris* extract and silver nanoparticles in controlling root-knot nematodes, the environmentally friendly feature of silver nanoparticles synthesized by the green method and its stability compared to plant extract, the objective of the current study was to determine the efficacy of as-synthesized AgNPs using *M. sylvestris* extract (Ms-AgNPs) on egg hatching and J2 mortality of *M. javanica* in vitro and on nematode-infected tomato under greenhouse conditions.

Materials and Methods

Microwave-assisted extraction (MWAE) of plant extract

M. sylvestris leaves were collected from rangelands in Shiraz, Iran, in 2019. The plant dried at 25 °C in the shade for ten days. Twenty grams of *M. sylvestris* leaves were dried, ground in a mortar, and soaked in 250 ml distilled water for 10 minutes. Microwave-assisted extraction (MWAE) was performed to obtain the aqueous leaf extract of mallow, using a microwave oven at 90 Watts, 20 min. Finally, the resulting mixture was filtered and stored at 4 °C (Moradi-Alvand *et al*., 2019b).

Synthesis of silver nanoparticles

For the plant-mediated synthesis of AgNPs, 50 ml of 0.05 M silver nitrate solution and 12.5 ml of ammonia solution were added to 50 ml of the obtained plant extract at 25 °C, with constant stirring under dark conditions. The obtained AgNPs were stored in the dark at 4 °C before experimental use (Vilchis-Nestor *et al*., 2008).

Characterization of silver nanoparticles

To confirm the production of silver nanoparticles, UV-vis spectra of the biosynthesis of Ms-AgNPs, plant extract, and ammonium were obtained at a 200–600 nm wavelength. For the synthesized silver nanoparticles, the peak of the plasmon surface is observed at 455 nm (Fig. 1a). The infrared bands of Ms-AgNPs were located at about 1081.44, 1385.37, 1616.77, 2925.73 and 3413.83 cm-1 (Fig. 1b). A TEM image of the nanoparticles was prepared to compare the size, morphology, and distribution of the nanoparticles. Optical properties of the as-prepared samples were determined using a UV-Vis absorption spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA) and a Fourier-transform infrared (FTIR) spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA). X-ray diffraction (XRD) of the samples was recorded using a Philips X'pert Pro (Philips, Amsterdam, Netherlands) with advanced diffractometer Cu Kα radiation of wavelength λ = 1.5406A°. Energy-dispersive X-ray (EDX) equipped with field emission scanning electron microscopy (FESEM) (Sigma-Aldrich Corp., St. Louis, MO, USA) was used to estimate the elemental composition and the mapping method, as well as the morphological analysis of the samples. The analysis of particle size was performed by transmission electron microscopy (TEM-EM 10 C) operated at 100 kV accelerating voltage (Zeiss, Oberkochen, Germany).

Egg hatchability assay

Ms-AgNPs were prepared at 0 (control), 62.5, 125, 250, 500, 1000 and 2000 ppm. Suspensions of each containing approximately 200 eggs of *M. javanica* were added separately to the 96-well microtiter tissue culture plates with a final volume of 250 µl of the experimental treatments and kept at 27 °C under dark conditions. The number of hatched eggs was counted after 96 hours. Each concentration (treatment) had four replicates, and the experiment was repeated twice.

J2s mortality assay

Ms-AgNPs were prepared at 0 (control), 10, 20, 60, 80, 100, 200, 400 and 800 ppm. Suspensions containing approximately 100 J2s were added to the 96-well microtiter tissue culture plates with a final volume of 250 µl of the experimental treatments and kept at 27 °C under dark conditions. The contents of each cell were added separately to sterile Petri dishes after 48 hours (Dourado *et al*., 2013), and 10 ml of sterile distilled water was added to remove Ms-AgNPs. After 24 hours, the mortality of J2 was assessed using a stereomicroscope based on their mobility (Meyer *et al*., 2006). Each concentration (treatment) had four replicates, and the experiment was repeated twice. Abbott's formula (1925) was used to determine the percentage of larval mortality (Equation 1).

$$\text{Percent of mortality}(\%) = \frac{\%\ \text{mortality in each treatment} - \%\ \text{mortality in control}}{100 - \%\ \text{mortality in control}}$$

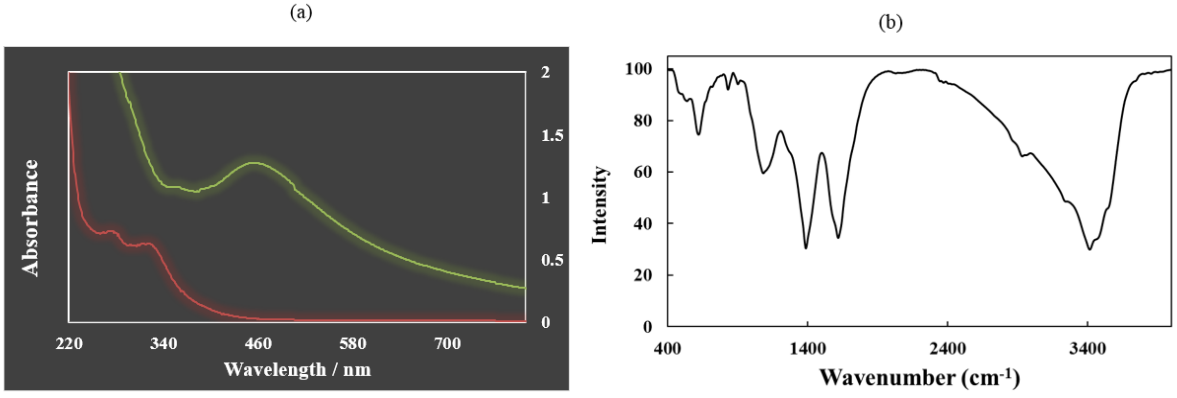

Figure 1 (a) UV–Vis absorption spectra of the plant extract and as-synthesized AgNPs and, (b) Fourier-transform infrared spectra of silver nanoparticles synthesized using *Malva sylvestris*.

Greenhouse experiments

Sandy-loam topsoil was steam-sterilized at 120°C for 150 minutes. Approximately one kg of soil was poured into plastic pots (10 cm in diameter). Seeds of tomato *Solanum lycopersicum*, cv. Early-Urbana (susceptible to *M. javanica*) were planted in plastic pots and maintained in a greenhouse (27 ± 3 °C temperature, under 16:8 hours (light: dark) photoperiod). Plants were watered daily; each received 20 ml 0.3% fertilizer (N-P-K, 20-20-20). Fertilization was applied eight times during the experiment. Four-leaf stage seedlings were separated into two sets; the first set was inoculated with three eggs/gram of soil, and the other was not inoculated. After three days, each set was soil-drenched (≈ 50 ml per plant) with 107, 164, and 250 ppm (equivalent to LC_{30}, $LC_{50,}$ and LC_{70}) of plant extract, AgNO3, and Ms-AgNPs. The experiment was arranged in a completely randomized design, and each treatment had five replicates. Experiments were repeated twice. After eight weeks, plants were harvested, and their growth indices were evaluated. Nematode population indices including the number of eggs in the root system (Hussey and Barker, 1973), galls and egg masses in the root system (root was stained with acid fuchsin) (Karssen, 2002) and the number of J2s in the soil (Whitehead and Hemming, 1965) were counted. Finally, the reproduction factor (RF) was calculated (Equation 2) (Taylor and Sasser, 1978).

Equation 2.

$$RF = \frac{\text{Final population of nematode (the numbers of nematode in soil and root system)}}{\text{Initial population of nematode}}$$

Statistical analysis

The data of both experiments were compared with the Tukey-Kramer HSD test at $P < 0.05$. The results were similar, and they were combined before statistical analysis. All data were subjected to a completely randomized design and statistically analyzed with SAS software (SAS Institute, Cary, NC) using the general linear model (GLM) procedure. The one-way analysis of variance (ANOVA) (egg hatching and the mortality tests), whereas the two-way ANOVA (the data of nematode population indices) and the three-way ANOVA (the data of plant growth indices) and pair-wise comparisons between treatment means were conducted using Tukey-Kramer HSD test at $P < 0.05$. To normalize the data, the percentages of egg hatching and the mortality rates were transformed into arcsin values (ArcSin√X) before statistical analysis. The relationship between concentration and percentage of lethal J2s was modeled by fitting a regression model (SAS, Proc Reg, Cary, NC).

Results

Characterization of silver nanoparticles

The color changed from light brown to sludge green within 24 hours after the start of the experiment, showing the reaction of the plant extract with silver nitrate (Fig. 2).

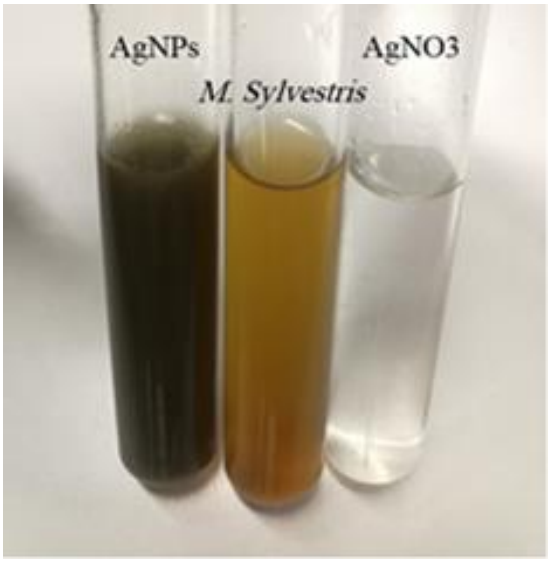

Figure 2 Color change of aqueous extract of *Malva sylvestris* after $AgNO_3$ addition and green synthesis of silver nanoparticles (Ms-AgNPs).

The X-ray diffraction spectrum (XRD) shows silver nanoparticles synthesized with an aqueous extract of *M. sylvestris* leaves. As can be seen, Bragg's reflections with 27.32°, 32.27°, 38.07° and 46.27° indexed to the peaks of 111, 200, 220, and 311 in the whole spectrum of 2θ values ranging from 10 to 80 are related to the Face-

centered cubic (FCC) structure of the silver nanoparticles (Fig. 3). The nanoparticles are spherically separated with an average diameter of 47.5 nm (Fig. 4).

EDX analysis also confirms that the synthesized silver nanoparticles are uniform in size and are spherical. The presence of the silver element in the mapping analysis demonstrated that the nanoparticles were synthesized and C, O, K, Cl, N, Ca, and Si are related to plant extract (Fig. 5).

Laboratory assay

The results showed that hatching significantly ($P < 0.05$) decreased with increasing concentration of Ms-AgNPs after 96 hours (Fig. 6). The mortality rate of J2s significantly ($P < 0.05$) increased with increasing concentration of Ms-AgNPs (Fig. 7).

Probit results of the effect of Ms-AgNPs on the percentage of J2s mortality were calculated (Table 1).

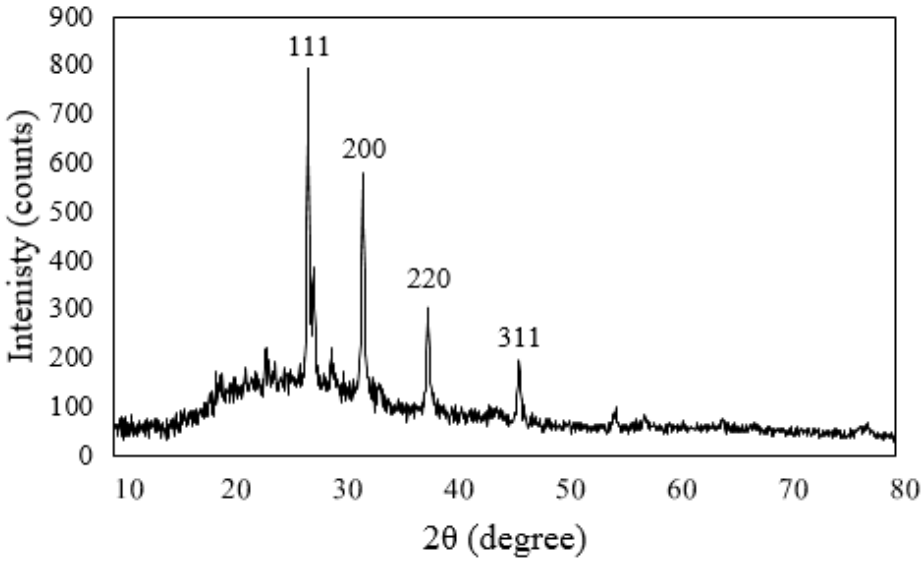

Figure 3 XRD pattern of as-synthesized AgNPs.

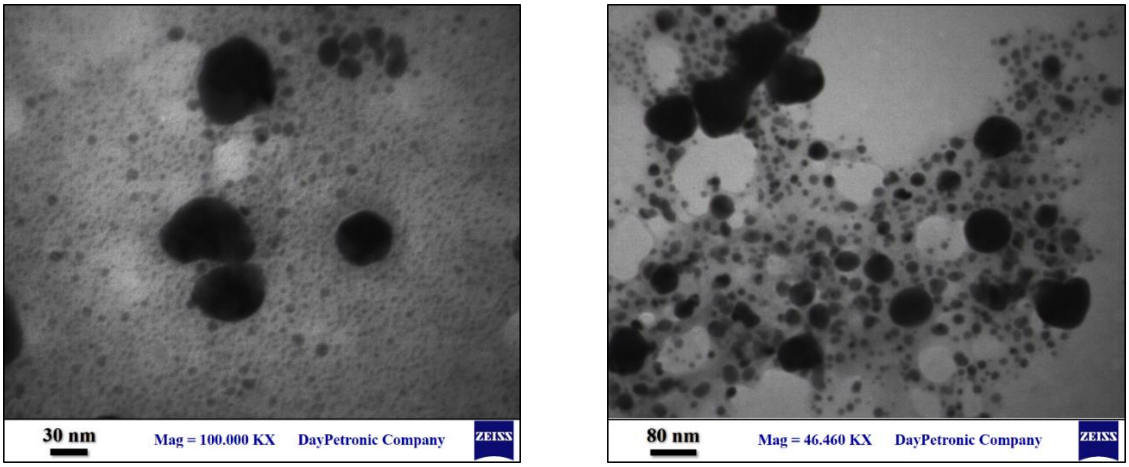

Figure 4 Transmission Electron Microscopy (TEM) images of as-synthesized AgNPs in two views.

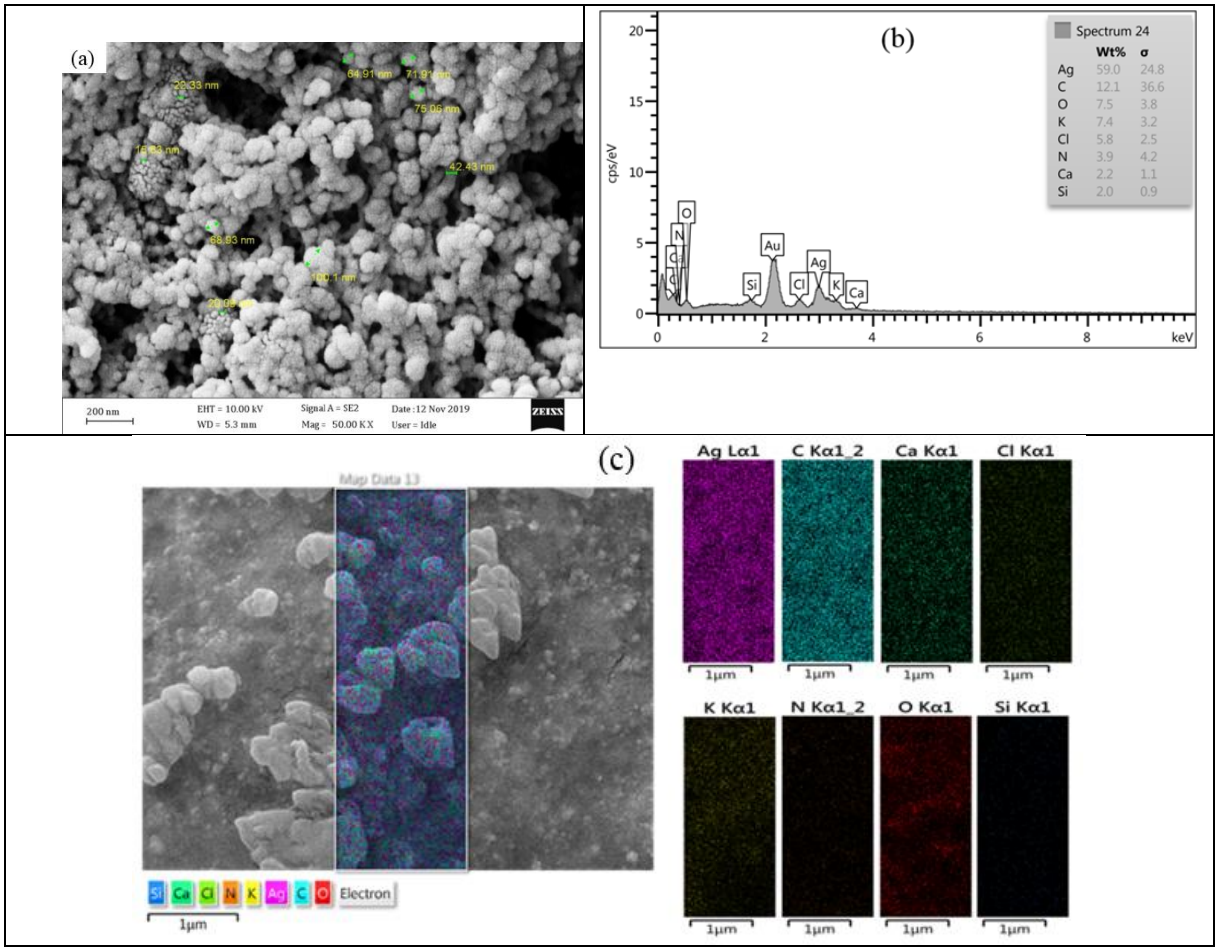

Figure 5 FESEM micrograph of silver nanoparticles synthesized using *Malva sylvestris*. (a) SEM image of Ms-AgNPs distribution, (b) the corresponding EDX elemental analysis and (c) Mapping analysis.

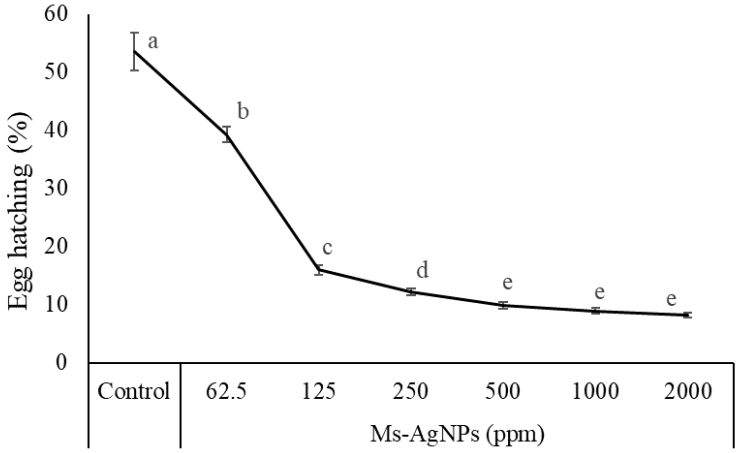

Figure 6 Mean egg hatching of *Meloidogyne javanica* 96 hours after exposure to different concentrations of synthesized silver nanoparticles using *Malva sylvestris* extract (Ms-AgNPs). Means are average of four replicates. Means with the same letter(s) are not different according to Tukey-Kramer HSD ($P \leq 0.05$).

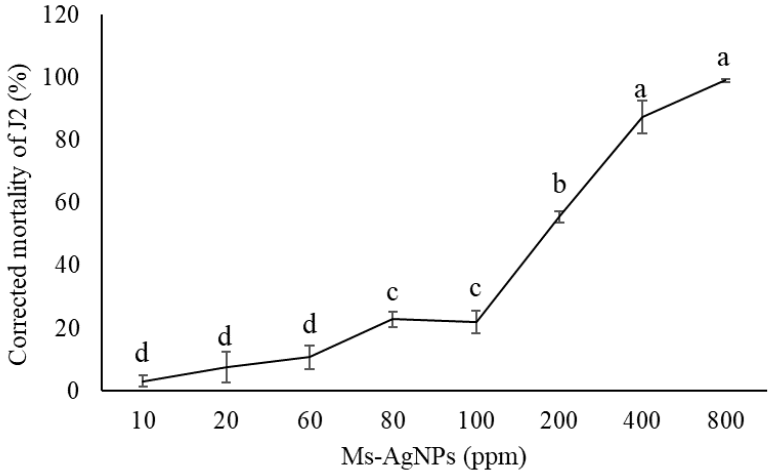

Figure 7 Corrected mortality rate of *Meloidogyne javanica* second-stage juveniles (J2) 48 hours after exposure to different concentrations of synthesized silver nanoparticles using *Malva sylvestris* extract (Ms-AgNPs) and 24 hours after transferring to distilled water. Means are average of four replicates. Means with the same letter(s) are not different according to Tukey-Kramer HSD ($p \leq 0.05$).

Table 1 Probit results of as-synthesized AgNPs using *Malva sylvestris* extract (Ms-AgNPs) on second-stage juveniles mortality of *Meloidogyne javanica*.

	Lethal concentration (ppm)					
	LC_{30}	LC_{50}	LC_{70}	R^2	Regression equation	Probability
Ms-AgNPs	107	164	250	0.99	Y=20848× - 1.257	0.01

Effect of Ms-AgNPs on infected tomatoes

The results showed that the treated plants with the highest Ms-AgNPs concentration had the lowest number of eggs/in the root system (Fig. 8a) and a significant reduction in the nematode reproduction rate ($P < 0.05$) (Fig. 8b). There was no significant difference in the number of galls/root system in plants treated with Ms-AgNPs (Fig. 8c). Ms-AgNPs at 250 ppm reduced the nematode reproduction factor and the number of eggs/root system by 56.3% and 60.4%, respectively, compared to untreated inoculated plants. Untreated inoculated plants showed the lowest number of egg masses/root systems, with no significant difference compared to plants treated with the highest concentration of plant extract, the lowest concentration of AgNO3, and all concentrations of Ms-AgNPs (Fig. 8d). There was no significant difference in the number of J2s in the plants treated with the *M. sylvestris* extract, $AgNO_3$ and Ms-AgNPs at all concentrations compared to the control plants (Figure 8e). Shoot length (Fig. 9a), shoot fresh weight (Fig. 9b), and shoot dry weight (Fig. 9c) of the inoculated plants did not change significantly compared to the non-inoculated plants in each treatment. Also, the fresh root weight of inoculated plants did not change significantly compared to the non-inoculated plants treated with different concentrations of Ms-AgNPs (Fig. 9d).

Discussion

The color change of the sample from light brown (plant extract) to sludge green (Ms-AgNPs) indicates the formation of silver nanoparticles. While no color change was observed with $AgNO_3$. The sludge green indicates the formation of a colloidal suspension of silver nanoparticles and a decrease in the ratio of Ag^+ to Ag ions. Silver nanoparticles show a sludge green color in an aqueous solution due to vibration-induced plasmonic surface oscillation

(Jain *et al*., 2009). The inhibitory property of the plant can be attributed to some secondary metabolites in the plant extract, such as flavonoids and phenols (Rajabi *et al*., 2017). UV-vis spectrophotometer was used to confirm the production of silver nanoparticles. This method can track the production of silver nanoparticles because of the surface plasmon resonance of the particles. Surface resonance is the common oscillation of electrons on the surface of metal nanostructures created by the response to an external stimulus such as light or an electric charge (Rastegarzadeh and Abdali, 2013). The maximum absorption peak in the curve obtained from the extract after the synthesis of silver nanoparticles in 400 to 460 nm indicates the synthesis of silver nanoparticles. According to previous reports, the maximum absorption of colloidal silver solution at wavelengths between 410 and 460nm was observed, which is attributed to the surface plasmon of different metal nanoparticles and the formation of quasi-linear nanoparticle structures (Mukherjee *et al*., 2001; Sharma *et al*., 2009). The plasmon resonance level of silver nanoparticles at 410-460 nm wavelength may be consistent with spherical nanoparticles (Rivallan *et al*., 2010).

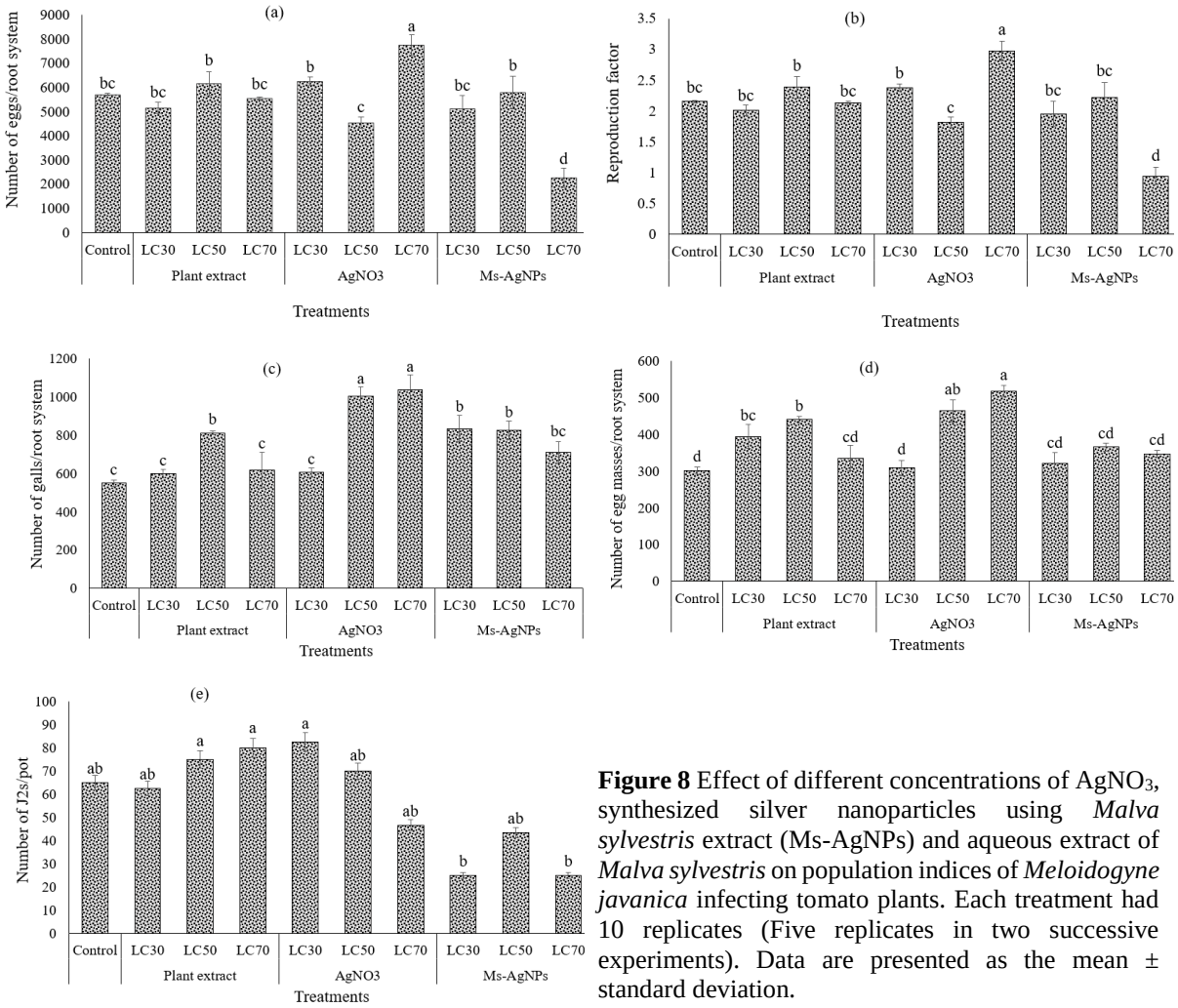

Figure 8 Effect of different concentrations of $AgNO_3$, synthesized silver nanoparticles using *Malva sylvestris* extract (Ms-AgNPs) and aqueous extract of *Malva sylvestris* on population indices of *Meloidogyne javanica* infecting tomato plants. Each treatment had 10 replicates (Five replicates in two successive experiments). Data are presented as the mean ± standard deviation.

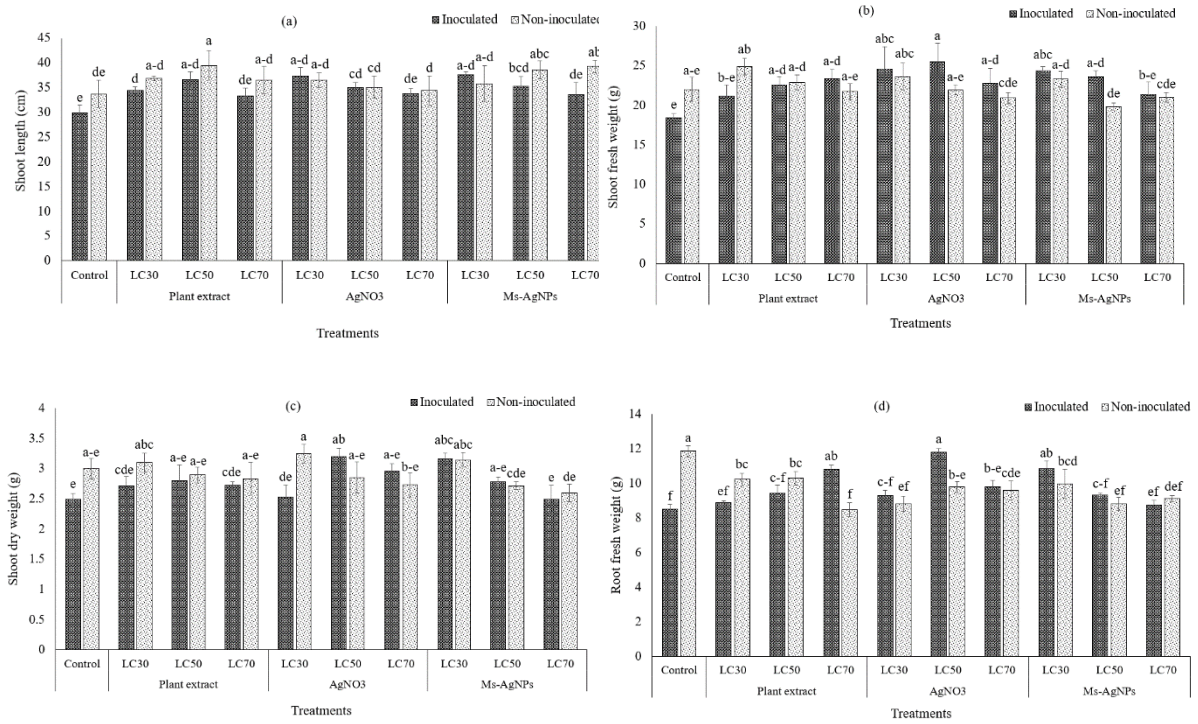

Figure 9 Effect of different concentrations of $AgNO_3$, Ms-AgNPs and aqueous extract of *Malva sylvestris* on growth of non-inoculated and inoculated tomato plants with *Meloidogyne javanica*. Each treatment had 10 replicates (Five replicates in two successive experiments). Data are presented as the mean ± standard deviation.

XRD shows the crystalline composition and organization of the synthesized nanoparticles by considering the size of the crystals in the sample (Ashour *et al*., 2018; Maksoud *et al*., 2019). X-ray diffraction spectra indicated that the AgNPs formed in this compound are crystalline. Our results are consistent with other reports that conform to the standard silver X-ray diffraction pattern (Baraka *et al*., 2017; El-Batal *et al*., 2018). The size difference between the formed silver nanoparticles is normal. Dwivedi *et al*. (2014) reported that physical and chemical properties, the shape, size, and size distribution of the nanoparticles depend on the quantity and quality of the plant material and the laboratory conditions. The presence of spherical, integrated, and small particles in the TEM image appears consistent with the UV-vis spectral study (Ahmad *et al*., 2003).

To find out the interaction between AgNPs and plant extract, FTIR measurements were calculated for the Ms-AgNPs (Niska *et al*., 2016). Results showed absorption peaks at about 1081.44, 1385.37, 1616.77, 2925.73 and 3413.83 cm^{-1}. Two strong, broad peaks were observed at 3413.83 and 3736.23 cm^{-1}. They correspond to the N−H stretching vibration of secondary amines. The band at 2925.73 cm^{-1} occurs due to the C−H stretching vibration of methylene, methyl, and methoxy groups. The absorption band at 1081.44 cm^{-1} was associated with C−O in ether. The band located at 1616.77 cm-1 corresponded to C=O bend vibration of ketones. The band at 1385.37 cm^{-1} is assigned for C=C. The band at 829.26 and 619.2 cm^{-1} is assigned to C−C. Our data showed the potential functional groups of ketone, ether, methyl, and C=C groups and their contribution to the capping of the Ms-AgNPs and reduction of silver ions (Kalaiselvi *et al*., 2019).

All used concentrations of *M. sylvestris* extract and $AgNO_3$ did not affect the reduction

of egg number and nematode reproduction factor. However, Ms-AgNPs significantly reduced the egg number and nematode reproduction factor in the treatment using LC_{70} equivalent concentration compared to the untreated control. The results showed that the use of Ms-AgNPs controls *M. javanica* nematode. The results of this study are consistent with the findings of Abdellatif *et al*. (2016), Nour El-Deen *et al*. (2014), El-Batal *et al*. (2018), and Kalaiselvi *et al*. (2019) findings that the use of green synthesized nanoparticles reduces the population and reproductive factor of nematodes. This is due to the presence of compounds in *M. sylvestris*, such as phenolics and flavonoids, especially antioxidants, with antioxidant, antimicrobial, and antiviral properties (Al-Rubaye *et al*., 2017; Hasimi *et al*., 2017) in Ms-AgNPs that are considered to be pre-disease inhibitors, providing a specific level of initial protection against bacteria, fungui, nematodes and pests (Sudhakar *et al*., 2007). Some plants also produce secondary compounds, including phenolic compounds essential as part of the plant's protection system against insects and pathogens such as root-knot nematodes (Wuyts *et al*., 2006). In addition, metal nanoparticles, by activating polyphenol oxidase (PPO) and peroxidase (POD) in tomato plants, play an important role in increasing resistance to plant pathogens (Morkunas and Gmerek, 2007). AgNPs interfere with ATP synthesis, membrane permeability, and response to oxidative stress (Choi and Hu, 2008; Lim *et al*., 2012).

The data showed that Ms-AgNPs did not significantly alter the growth indices of treated tomato plants compared with control plants. The present results contradict those of Surega (2015) on tomato plants and Abdellatif *et al*. (2016) on eggplant, who found that the synthesized nanoparticles increased plant vegetative parameters. Reduction of the reproductive factor of less than one (0.94) at the highest concentration of silver nanoparticles indicates that it declines after an initial breeding season and decreases yearly after that. Molinari and Baser (2010) showed that treatment with certain chemicals increased plant activity and production of defense enzymes and produced an additional load for the plant, reducing plant vegetative parameters. In this study, using Ms-AgNPs without imposing overload on the plant has reduced the nematode population.

Conclusion

This study showed that Ms-AgNPs have a high potential to reduce the population of *M. javanica* in infected tomato plants and not overload the plant. On the other hand, plant extract and silver nitrate showed the least efficacy in nematode control, proving that the nanoparticles synthesized with these two materials play a more effective role than nematode control when used alone. The findings of this study indicate that the synthesized nanoparticles can be used in greenhouse tomato cultivation to reduce the nematode population. This paper describes a novel approach for the control of root-knot nematodes. Such a technique is a safe and effective management program for nematode control. Evaluation of the effects of Ms-AgNPs on tomatoes in fields naturally infested with root-knot nematodes is suggested.

Acknowledgments

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Declarations

Conflict of Interest

The authors declare no conflict of interest.

References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Abdellatif, K. F., Abdelfattah, R. H. and El-Ansary, M. S. 2016. Green nanoparticles engineering on root-knot nematode infecting eggplants and their effect on plant DNA

modification. Iranian Journal of Biotechnology, 14: 250-259.

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, I. M., Kumar, R. and Sastry, M. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B: Biointerfaces, 28: 313-318.

Alikarami, M., Charehgani, H. and Abdollahi, M. 2017. Nematicidal activity of some plant extracts on root-knot nematode on tomato (*Solanum lycopersicum*) *in vitro* and *in vivo* conditions. Iranian Journal of Plant Protection Science, 48: 317-326.

Al-Rubaye, A. F., Kaizal, A. F. and Hameed, I. H. 2017. Phytochemical screening of methanolic leaves extract of *Malva sylvestris*. International Journal of Pharmacognosy and Phytochemical Research, 9: 537-552.

Ashour, A., El-Batal, A. I., Maksoud, M. A., El-Sayyad, G. S., Labib, S., Abdeltwab, E. and El-Okr, M. 2018. Antimicrobial activity of metal-substituted cobalt ferrite nanoparticles synthesized by sol–gel technique. Particuology, 40: 141-151.

Baraka, A., Dickson, S., Gobara, M., El-Sayyad, G. S., Zorainy, M., Awaad, M. I., Hatem, H., Kotb, M. M. and Tawfic, A. 2017. Synthesis of silver nanoparticles using natural pigments extracted from Alfalfa leaves and its use for antimicrobial activity. Chemical Papers, 71: 2271-2281.

Choi, O. and Hu, Z. 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environmental Science and Technology, 42: 4583-4588.

Crow, W. T., Luc, J. E. and Giblin-Davis, R. M. 2011. Evaluation of econemTM, a formulated *Pasteuria* sp. bionematicide, for management of *Belonolaimus longicaudatus* on golf course turf. Journal of Nematology, 43: 101-109.

Dourado, D. P., Lima, F. S. and Muraishi, C. T. 2013. Nematicidal activity *in vitro* and *in vivo* of neem oil on *Meloidogyne incognita*. Brazilian Journal of Applied Technology for Agricultural Science, 6: 63-68.

Dwivedi, P., Narvi, S. S. and Tewari, R. P. 2014. Phytofabrication characterization and comparative analysis of Ag nanoparticles by diverse biochemicals from *Elaeocarpus ganitrus* Roxb., *Terminalia arjuna* Roxb., *Pseudotsuga menzietii*, *Prosopis spicigera*, *Ficus religiosa*, *Ocimum sanctum*, *Curcuma longa*. Industrial Crops and Products, 54: 22-31.

El-Batal, A. I., Mosallam, F. M. and El-Sayyad, G. S. 2018. Synthesis of metallic silver nanoparticles by fluconazole drug and gamma rays to inhibit the growth of multidrug-resistant microbes. Journal of Cluster Science, 29: 1003-1015.

Fernandez, C., Rodriguez-Kabana, R., Warrior, P. and Kloepper, J. W. 2001. Induced soil suppressiveness to a root-knot nematode species by a nematicide. Biological Control, 22: 103-114.

Hasimi, N., Ertaş, A., Oral. E. V., Alkan, H., Boğa, M., Yılmaz, M. A., Yener, I., Gazioğlu, I., Özaslan, C., Akdeniz, M. and Kolak, U. 2017. Chemical profile of *Malva neglecta* and *Malvella sherardiana* by Lc-MS/MS, GC/MS and their anticholinesterase, antimicrobial and antioxidant properties with aflatoxin-contents. Marmara Pharmaceutical Journal, 21: 471-484.

Hussey, R. S. and Barker, K. R. 1973. A comparision of methods of inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 57: 1025-1028.

Jacob, S. J., Finub, J. S. and Narayana, A. 2012. Synthesis of silver nanoparticles using *Piper longum* leaf extracts and its cytotoxic activity against Hep-2 cell line. Colloids Surf B Biointerfaces, 91: 212-214.

Jain, D., Kumar, D.H., Kachhwaha, S. and Kothari, S. 2009. Synthesis of plant-mediated silver nano particles using papaya fruit extract and evaluation of their antimicrobial activities. *Digest* Journal of Nanomaterials and Biostructures, 4: 723-727.

Kalaiselvi, D., Mohankumar, A., Shanmugam, G., Nivitha, S. and Sundararaj, P. 2019. Green synthesis of silver nanoparticles using latex extract of *Euphorbia tirucalli*: A novel

approach for the management of root knot nematode, *Meloidogyne incognita*. Crop Protection, 117: 108-114.

Karssen, G. 2002. The plant-parasitic nematode genus *Meloidogyne* Göldi, 1892 (Tylenchida) in Europe. 6th edition, Leiden-Netherlands: Brill.

Khan, A., Mohd, A., Moh, T., Bushra, R., Kavita, P. and Mansoor, A. S. 2017. Phytochemical investigation nematostatic and nematicidal potential of weeds extract against the root-knot nematode *Meloidogyne incognita* in vitro. Asian Journal of Biological Sciences, 10: 38-46.

Lara, H. H., Garza-Trevino, E. N., Ixtepan-Turrent, L. and Singh, D. K. 2011. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. Journal of Nanobiotechnology, 9: 30-37.

Lim, D., Roh, J. Y., Eom, H. J., Hyun, J. W. and Choi, J. 2012. Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. Environmental Toxicology and Chemistry, 31: 585-592.

Lust, J. 2014. The Herb Book: The Most Complete Catalog of Herbs Ever Published. Mincola, New York: Dover Publications.

Maksoud, M. A., El-Sayyad, G. S., Ashour, A., El-Batal, A. I., Elsayed, M. A., Gobara, M., El-Khawaga, A. M., Abdel-Khalek, E. and El-Okr, M. 2019. Antibacterial, antibiofilm, and photocatalytic activities of metals-substituted spinel cobalt ferrite nanoparticles. Microbial Pathogenesis, 127: 144-158.

Meyer, S. L. F., Zasada, I. A., Roberts, D. P., Vinyard, B. T., Lakshman, D. K., Lee, J., Chitwood, D. J. and Carta, L. K. 2006. *Plantago lanceolata* and *Plantago rugelii* extracts are toxic to *Meloidogyne incognita* but not to certain microbes. Journal of Nematology, 38: 333-338.

Mirsadeghi, S., Koudehi, M. F., Rajabi, H. R. and Pourmortazavi, S. M. 2019. Green and simple synthesis of silver nanoparticles by aqueous extract of *Perovskia abrotanoides*: Characterization, optimization and antimicrobial activity. Current Pharmaceutical Biotechnology, https://doi.org/10.2174/1389201020666190618121218.

Mittal, A. K., Chisti, Y. and Banerjee, U. C. 2013. Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, 31: 346-356.

Molinari, S. and Baser, N. 2010. Induction of resistance to root-knot nematodes by SAR elicitors in tomato. Crop Protection, 29: 1354-1362.

Moradi-Alvand, Z., Rajabi, H. R., Mirzaei, A. and Masoumi, A. 2019a. Ultrasonic and microwave assisted extraction as rapid and efficient techniques for plant mediated synthesis of quantum dots: Green synthesis, characterization of zinc telluride and comparison study of some biological activities. New Journal of Chemistry, 43: 15126-15138.

Moradi-Alvand, Z., Rajabi, H. R., Mirzaei, A., Masoumi, A. and Sadatfaraji, H. 2019b. Rapid and green synthesis of cadmium telluride quantum dots with low toxicity based on a plant-mediated approach after microwave and ultrasonic assisted extraction: Synthesis, characterization, biological potentials and comparison study. Materials Science and Engineering, 98: 535-544.

Morkunas, I. and Gmerek, J. 2007. The possible involvement of peroxidase in defense of yellow lupine embryo axes against *Fusarium oxysporum*. Journal of Plant Physiology, 164: 185-194.

Mukherjee, P. A., Ahmad, D., Mandal, S., Senapati, S., Sainkar, S. R., Khan, M. I., Parishcha, R., Ajaykumar, P. V., Alam, M., Kumar, R. and Sastry, M. 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Letters, 1: 515-519.

Nassar, A. M. 2016. Effectiveness of silver nanoparticles of extracts of *Urtica urens* (Urticaceae) against root-knot nematode *Meloidogyne incognita*. Asian Journal of Nematology, 5: 14-19.

Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S. and Dhakshnamoorthy, G. 2006. Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. Crop Protection, 25: 1210-1213.

Niska, K., Knap, N., Kędzia, A., Jaskiewicz, M., Kamysz, W. and Inkielewicz-Stepniak, I. 2016. Capping agent-dependent toxicity and antimicrobial activity of silver nanoparticles: an *in vitro* study. Concerns about potential application in dental practice. International Journal of Medical Sciences, 13: 772-782.

Nour El-Deen, A. H., Cseh, E. and Darwesh, H. Y. 2014. Evaluation of certain Hungarian plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita* in-vitro. International Journal of Advanced Research, 2: 443-448.

Osaka, T., Matsunaga, T., Nakanishi, T., Arkaki, A., Niwa, D. and Iida, H. 2006. Synthesis of magnetic nanoparticles and their application to bioassays. Analytical Bioanalytical Chemistry, 384: 593-600.

Peng, S., Wang, C., Xie, J. and Sun, S. 2006. Synthesis and stabilization of monodisperse Fe nanoparticles. Journal of the American Chemical Society, 128: 10676-10677.

Rafiee, F., Charehgani, H. and Abdollahi, M. 2019. Evaluation of burdock and Mountain almond leaf extracts against *Meloidogyne javanica* on tomato. Archives of Phytopathology Plant Protection, 52: 1035-1047.

Rajabi, H. R., Naghiha, R., Kheirizadeh, M., Sadatfaraji, H., Mirzaei, A. and Moradi-Alvand, Z. 2017. Microwave assisted extraction as an efficient approach for biosynthesis of zinc oxide nanoparticles: Synthesis, characterization, and biological properties. Materials Science and Engineering C, 78: 1109-1118.

Rajeshkumar, S., Malarkodi, C., Gnanajobitha, G., Paulkumar, K., Vanaja, M., Kannan, C. and Annadurai, G. 2013. Seaweed-mediated synthesis of gold nanoparticles using *Turbinaria conoides* and its characterization. Journal of Nanostructure in Chemistry, 3: 44-50.

Rastegarzadeh, S. and Abdali, Sh. 2013. Colorimetric determination of thiram based on formation of gold nanoparticles using ascorbic acid. Talanta, 104: 22-26.

Rivallan, M., Thomas, S., Lepage, M., Takagi, N., Hirata, H. and Thibault-Starzyk, F. 2010. Evolution of platinum particles dispersed on zeolite upon oxidation catalysis and ageing. ChemCatChem, 2: 1599-1605.

Roh, J. Y., Sim, S. J., Yi, J., Park, K., Chung, K. H., Ryu, D. Y. and Choi, J. 2009. Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. Environmental Science and Technology, 43: 3933-3940.

Sadeghi, A., Soltani, B.M., Salehi Jouzani, G., Karimi, E., Khayam Nekouei, M. and Sadeghizadeh, M. 2014. Taxonomic study of a salt tolerant *Streptomyces* sp. strain C- 2012 and the effect of salt and ectoine on lon expression level. Microbiological Research, 169: 232-238.

Sadeghi, B., Rostami, A. and Momei, S. S. 2015. Facile green synthesis of silver nanoparticles using seed aqueous extract of *Pistacia atlantica* and its antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 134: 326-332.

Senapati, S., Syed, A., Moeez, S., Kumar, A. and Ahmad, A. 2012. Intracellular synthesis of gold nanoparticles using alga *Tetraselmis kochinensis*. Materials Letters, 2: 275-281.

Sharma, V.K., Yngard, R.A. and Lin, Y. 2009. Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145: 83-96.

Singh, N. G., Anil, K., Sewak, R. and Vinod, K. 2017. Evaluation of nematicidal activity of ethanolic extracts of medicinal plants to *Meloidogyne incognita* (Kofoid and White) Chitwood under lab conditions. International Journal of Pure and Applied Bioscience, 5: 827-831.

Singh, P. K., Bhardwaj, K., Dubey, P. and Prabhune, A. 2015. UV-assisted size sampling and antibacterial screening of *Lantana camara*

leaf extract synthesized silver nanoparticles. RSC Advaces, 5: 24513-24520.

Sudhakar, N., Nagendra-Prasad, D., Mohan, N. and Murugesan, K. 2007. Induction of systemic resistance in *Lycopersicon esculentum* cv. PKM1 (tomato) against *Cucumber mosaic virus* by using ozone. Journal of Virological Methods, 139: 71-77.

Surega, R., Ramakrishnan, S., Anita, B., Gunasekaran, K. and Nakkeeran, S. 2015. Antifungal effect of plant mediated silver nanoparticles against *Fusarium oxysporum* f.sp. *lycopersici*. International Journal of Tropical Agriculture, 33: 157-160.

Taylor, A. L. and Sasser, J. N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh, North Carolina, USA: North Carolina State University Graphics.

Thakore, S. I., Nagar, P. S., Jadeja, R. N., Thounaojam, M., Devkar, R. V. and Rathore, P.S. 2019. Sapota fruit latex mediated synthesis of Ag, Cu mono and bimetallic nanoparticles and their in vitro toxicity studies. Arabian Journal of Chemistry, 12: 694-700.

Vilchis-Nestor, A. R., Sánchez-Mendieta, V., Camacho-López, M. A., Gómez-Espinosa, R. M., Camacho-López, M. A. and Arenas-Alatorre, J. A. 2008. Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. Materials Letters, 62: 3103-3105.

Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting vermiform nematodes from soil. Annals of Applied Biology, 55: 25-38.

Wuyts, N., Waele, D. D. and Swennen, R. 2006. Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande naine) roots. Plant Physiology and Biochemistry, 44: 308-314.

سنتز سبز نانوذرات نقره با استفاده از گیاه پنیرک*Malva sylvestris* به‌عنوان یک راهکار مدیریتی بالقوه برای کنترل نماتد ریشه‌گرهی *Meloidogyne javanica* در گوجه‌فرنگی *Solanum lycopersicum*

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چکیده: نماتدهای ریشه‌گرهی، (*Meloidogyne* spp.)، عامل کاهش پنج درصدی تولید محصولات گیاهی در جهان هستند. نانوذرات نقره (AgNPs) یک نماتدکش بالقوه می‌باشند. روش‌های زیادی برای تولید نانوذرات فلزی، ازجمله استفاده از ترکیبات آلی بی‌خطر برای محیط‌زیست وجود دارد. در مطالعه حاضر، کارایی نانوذرات نقره سنتز شده با استفاده از عصاره گیاه پنیرک (Ms-AgNPs) روی میزان تفریخ تخم و مرگ‌ومیر لارو سن دو نماتد *M. javanica* در شرایط آزمایشگاهی و روی گوجه‌فرنگی آلوده به نماتد در شرایط گلخانه مورد بررسی قرار گرفت. نتایج نشان داد که افزایش غلظت Ms-AgNPs باعث کاهش میزان تفریخ تخم و افزایش میزان مرگ‌ومیر لارو سن دو نماتد گردید. Ms-AgNPs در غلظت‌های ۱۰۷، ۱۶۴ و ۲۵۰ قسمت در میلیون، به‌ترتیب باعث کاهش ۳۰، ۵۰ و ۷۰ درصدی لاروهای سن دو نماتد شده و به‌عنوان دزهای کشنده نماتد مشخص شدند. Ms-AgNPs در غلظت ۲۵۰ قسمت در میلیون باعث کاهش تعداد تخم نماتد در سیستم ریشه و فاکتور تولیدمثل به‌ترتیب به‌میزان ۶۰/۴ و ۵۶/۳ درصد و باعث افزایش طول و وزن تر شاخساره به‌ترتیب به‌میزان ۱۱/۲ و ۱۴ درصد در مقایسه با گیاهان شاهد شدند. Ms-AgNPs در پایین‌ترین غلظت، بدون ایجاد آثار منفی روی گوجه‌فرنگی، باعث کاهش جمعیت نماتد *M. javanica* شد و با توجه به این‌که ترکیبی بی‌خطر برای محیط‌زیست می‌باشد، می‌توان از آن برای مدیریت نماتدهای انگل گیاهی استفاده کرد.

واژگان کلیدی: استخراج به‌کمک مایکروویو، سنتز با استفاده از گیاهان، کنترل