Nematicidal Activity of Silver Nanoparticles Synthesized by Seaweeds Extracts Against Meloidogyne Incognita on Tomato Plant

Rehab Y. Ghareeb
City of Scientific Research and Technological Applications
Nihal Galal El-Din Shams El-Din
City of Scientific Research and Technological Applications
Dina S. S. Ibrahim
agriculture research center
Bandar S. Aljuaid
Taif University
Ahmed M. Elshehawi
Taif University
Ahmed Abdel-Megeed
Alexandria University
Nader Abdelsalam (nader.wheat@alexu.edu.eg)
Alexandria University

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Abstract

This study aimed to evaluate the nematicidal activity of two marine algae (*Colpomenia sinuosa* and *Corallina mediterranea*) extracts and their synthesized silver nanoparticles against the root-knot nematode (*Meloidogyne incognita*) infecting tomato plant. Scanning Electron Microscope (SEM) showed that the obtained nanoparticles were aggregated in anisotropic Ag particles. Transmission Electron Microscope (TEM), results showed the particles size was less than 40 nm. Whenever, FT-IR analysis spectrum presented sharp absorbance between 440 and 4000 cm-1 for the obtained nanoparticles, with 13 distinct peaks ranged from 3915 – 474.Both of methylene chloride extract and its synthesized green silver nanoparticles were applied against the M. incognita. The results indicated that the synthesized silver nanoparticles of *C. sinuosa* exhibited the highest nematicidal activity. Besides, they reduced number of nematode galls, number of egg-masses per root and eggs/egg mass, as well as growth parameters of the treated plants with nanoparticles were enhanced comparing with the other treatments. While the methylene chloride extract of *C. sinuosa* exhibited higher activity than that of *C. mediterranea*, and the most effective eleuent of this solvent was Hexane: methylene chloride: ethyl acetate (1: 0.5: 0.5, v/v/v). The 3rd fraction of this eluent was the most effective one when it was applied on M. incognita, resulting in 87.5 % mortality after 12 h and 100 % after 24 and 72 h of exposure. The analysis of this fraction revealed the presence of seven bioactive constituents. Conclusively, the synthesized silver nanoparticles of *C. sinuosa* could be considered as alternative chemical nematicides.

1. Introduction

Plant-Parasitic Nematodes (PPN) cause significant damage and losses to most of agricultural crops in the tropical and sub-tropics regions [1,2], which are estimated to be annually $100 billion worldwide [3,4]. The root- knot nematode (Meloidogyne spp.) is widespread, attack a broad species of crops. It was reported that more than 3000 species of hosts which causes serious damage to most agricultural crops world-wide [4,5]. The four common root nematodes species, *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are the most enormous and crop destructive nematodes [4,6].

Plant-pathogenic nematodes (Root-knot nematodes) have been found to cause crop yield losses of 8.8 % in developed countries while losses of 14.6% have been observed in tropical and subtropical countries, moreover, they invaded an array of important crops and have been found more damaging to vegetables [7,8]. In the absence of effective control, *M. incognita* can cause total crop failure. Tomato (*Solanum lycopersicum*) is the most important vegetable crop widely used throughout the world and is often susceptible to the attack by Meloidogyne spp., which limits the quantity and quality of fruit yield [9,10].

Several studies reported the damage is potentially different caused by Meloidogyne spp. on different tomato cultivars under pot, microplot and field experiment conditions throughout the world [11]. Yield losses of 22–30 % have been reported on tomato resulted in *M. incognita* infection. Yield loss in tomato

due to root-knot nematode has been estimated to be up to 61.0% [12]. Meanwhile, damage ranged from 32 to 40% as reported by [13].

The chemical pesticides are typically used for nematode controls. Noticeably, the overuse of such pesticides has resulted in a harmful environmental effect [14]. Moreover, the extensive use of nematicides in controlling of nematodes which led to environmental and health problems and mainly increased nematode resistance. Accordingly, alternative methods of root-knot nematode management which are environmentally friendly and cost-effective are of great interest to researchers and plant breeders.

Recently, nanotechnology has been successfully applied to pest management infected plant crops [2,4,15], in addition, the use of silver nanoparticles (AgNPs) has been shown to clarify anti-nematode impacts [16]. Silver nanoparticles are often synthesized by using strong and complicated chemicals [17]. Thus, a considerable number of recent studies extensively focused on the feasibility of biological synthesis of environment friendly, non-toxic nanocomposites [18,19].

The use of biological materials including plant extract, fungi, bacteria, and seaweeds for the synthesis of nanoparticles showed numbers of benefits of eco-friendliness and compatibility for pharmaceuticals and other biomedical applications, as they avoid the toxic chemicals for the synthesis process [4,18,20]. Among all species of algae, Chlorophyta, Phaeophyta and Rhodophyta considered most important and major groups [21]. These massive varieties of seaweeds (marine algae) found to possess useful untapped biochemical compounds such as carotenoids, dietary fibers, fatty acids which might be potential source of pest control [22], In addition, the marine algae have a wide range of compounds such as agar, acids, carotenes, alkaloids, and phenolic compounds. Some of these compounds have pesticides activity [23]. Antibiotics, such as bromo-phenols, tannins, phloroglucinol, and terpenoids, have anti-nematodes activity [24].

Alginate isolation from marine brown algae is a common type of crystallizing agent used in food industries, medicine, and plant pest biocontrol [25]. The main advantages of alginate preparations are non-toxic nature, fast degradation, and release of microorganisms into the soil [26].

Algal alginates extracted from *Colpomenia sinuosa*, was tested against the *M. javanica* infecting eggplant (*Solanum melongena* L.) under green-house conditions, results revealed that these remarkable compounds significantly reduce both the development and reproduction of the *M. javanica* and worked in increase the growth parameters of eggplant (infected with nematode) compared to untreated infected plants [27].

Red seaweeds Corallina sp. could be review as a prospective source of bioactive molecules such as minerals and saturated fatty acids, sulfated galactans and carrageenan which displayed antimicrobial activity [28], also, they tested different algae for their nematicidal activity against *M. incognita* infected tomato plants; it was observed that *Corallina officinalis, C. mediteranea* and *Ulva fasciata* showed nematicidal activity against the root knot nematode *M. incognita*.

Recently, the development of nanotechnologies in many fields such as biology, medicine, pharmacology and as well as agriculture gave rise to the application of green silver nanoparticles as a new approach to control root knot nematodes [29,30]. Khan et al. [31] reported that different seaweeds exhibited significant nematicidal activities such as: inhibition of egg hatching, increasement larval mortality and reduction of root-knot disease.

Sequency the present study was conducted to evaluate the efficiency of either *Copromania sinuosa* and *Corallina mediterranean* extracts or the synthesized green silver nanoparticles as a nematicidal activity against second-stage juveniles and mortality of *M. incognita* on tomato crop. Moreover, to prospect the strongest bioactive compounds in the two examined algal extracts which showed a high potency as nematicides.

2. Materials And Methods

The selected tomato plants are officially collected from the in-house Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Borg El-Arab, Alexandria, Egypt Farm which is allocated for Research and Developments only.

2.1. Study area

Algal samples were collected in June 2015 from two stations on the coastal line of Alexandria, Egypt, namely, Abu-Qir (31°19′26″N, 30°3′41″E) and Gleem seashore (31°15′28″N, 29°57′28″E).

2.2. Collection of macroalgae

Algal samples were collected by hand at the sub-littoral zone (0.5-1 m depth), washed with seawater at the sampling site to remove the adhered sediments and impurities then separated in polyethylene bags. Samples were stored in ice box at 4°C. Quick rinsing of the collected algae using tap water was carried out in the laboratory on the same day to get rid of the remaining impurities and epiphytes. Microscopic investigation of a whole collected mount of each algal species was carried out and morphological identification was performed according to [32,33].

The two collected algal species were identified. The first alga collected from Abu-Qir was identified as *Colpomenia sinuosa* (Mertens ex Roth) Derbes et Solier belonging to the class Phaeophyceae, order Scytosiphonales, family Scytosiphonaceae. The 2nd alga collected from Gleem seashore was identified as *Corallina officinalis* Linnaeus be-longing to the class Rhodophyceae, order Corallinales, family Corallinaceae. About 250 g of each representative species at each study area were air dried to constant weight at room temperature (25°C). Thereafter, the dried alga was milled in an electric blender into a fine powder.

2.3. Source of root knot nematode

Nematode inoculum was extracted from pure cultures of *M. incognita* maintained in the greenhouse of City of Scientific Research and Technological Application, Alexandria, Egypt, on black nightshade, *Solanum nigrum* Lin. (Solanaceae). For the extraction, galled roots were collected and washed using tap water to remove the adhering soil particles and the egg masses in the galls were collected under stereoscopic microscope (LABOMED; Labo America, Inc. USA) with a needle.

The egg masses were incubated in Petri dish with distilled water for 48 h at room temperature at 27 ± 2°C for hatching, thereafter, eggs hatched into active second-stage juveniles (J2S) were collected. Inoculum of 3000 root knot nematode juveniles/pot were added into one month old tomato plants cv. Alisa, planted in 2.5 kg of sterilized sandy/clay soil mixture in the greenhouse at 27 ± 2°C [34,35].

After 35 days from nematode inoculation, the eggs were extracted from the galled roots washed and cut into 1 cm long, shaken for three minutes in 1 liter of 0.5 % of sodium hypochlorite solution (NaOCl) to extract nematodes eggs [36,37]. The obtained egg suspension was sieved through 200 and 500 mesh sieves. The nematode eggs retained on 500 mesh opening sieve were collected in 100 ml plastic beakers. Nematode eggs were allowed to hatch in sterile distilled water at 26 ± 3°C and newly hatched second-stage juveniles (J2S) were collected. Freshly hatched J2S which were collected were used as nematode inoculum.

2.4. Morphological identification of root knot nematode

According to [38], ten mature females of Meloidogyne spp. were removed from the root tissue by detaching apart with forceps and remove adult females. Females were separated from the egg masses and placed in a drop of warm lactophenol on a clear glass slide for perennial pattern identification using a light microscope [39].

2.5. Preparation of the two macroalgae extracts

Different analytical grade organic solvents were used to prepare algal extracts from the two collected species *C. sinuosa* and *C. mediterranea* to choose the most effective solvent. This was performed by fractionation of algal crude extract [40]. Five grams powder from each dried algal species were extracted with 50 ml n-hexane (1:10 w: v) and shaken at 150 rpm overnight (IKA®-WERKE, Germany). The extracted solution was centrifuged at 10000 g (Hermal labortechnlk, Gmbh. Germany) for 15 min to collect the supernatant. The extract was separated from the alga by filtration, using filter paper (GVS, 125mm). After hexane extraction, the seaweed sample was air dried in a ventilated place at ambient temperature of 25 ± 2°C until the constant weight was reached.

The air-dried residue was then extracted successively 3 times by using separately the organic solvents (methylene chloride, ethyl acetate and finally n-butanol), following the same procedure of the first extraction. The four supernatants obtained from the extraction process of each algal species were used each separately in the bioassay tests on the nematode species M. incognita, where the methylene chloride extract exhibited the highest activity for both algae in the bioassay. Therefore, five grams of the algal dry weight (powder) either from *C. sinuosa or C. mediterranea* were dispersed in 100 ml methylene

chloride, with shaking at 150 rpm/45°C overnight. The extracts were filtered through Millipore filter (0.2 µm) and stored at -20°C for further studies.

2.6. Biosynthesis of Ag-NPs using the macroalgae extract

The silver nanoparticles (Ag-NPs) were biosynthesized as described by [41], with slight modifications. The Erlenmeyer flask containing 100 ml of aqueous solution (1 mM) of AgNO3 was mixed with 100 ml of the aqueous extract of either *C. sinuosa or C. mediterranea* for (1 h) under continuous stirring at 40°C, then allowed to stand for (1 h) at room temperature (25°C). The color of the reaction mixture gradually turned to dark brown from transparent yellow, indicating the formation of Ag-NPs. The synthetic reaction was completed in 2 h. The initial pH of the solution was approximately 7.5 but changed to 5.6 by the end of the reaction. The dark brown solid product was collected through centrifugation at 11000 g for 12 min and washed with distilled water from five to ten times. The final pellet was left at 35°C to dry. The dried sample was deposited with a few drops of ethanol and grinded into powder and stored for further analysis.

2.7. Purification of synthesized silver nanoparticles

Bio-synthesized silver nanoparticles were purified by distilled water and 70% ethanol by repeated centrifugation at 5000 g for 20 min.

2.7.1. Characterization of Ag-NPs using Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) examination was performed to visualize the color changes of the synthesized Ag-NPs and their morphology, which were harvested by centrifugation at 8000 g for 15 min at 4°C, then washed with absolute ethanol and fixed with 2% glutaraldehyde followed by 1% osmium tetroxide (OsO4). After complete fixation, the samples were washed with absolute ethanol, and then dehydrated in ascending order of ethanol concentrations (50, 75 and 100%). The fixed Ag-NPs were then dried completely and finally coated with thin layer of gold. The average particle size of Ag-NPs was determined by measuring and averaging the particle size of approximately from 25 to 55 nm random particles in each sample, using the SEM software (SMILE VIEW SOFTWARE developed by JOEL on Scanning Electron Microscope- JEOL JSM-6490) after sputtering by gold.

2.7.2. Characterization of Ag-NPs using Transmission Electron Microscope (TEM)

Samples of synthesis silver nanoparticles were prepared for TEM observation test by dispersing small quantities of the dried sample into distilled water and followed by depositing a few drops of the resulted suspension on a copper grid (Field Emission Transmission Electron Microscope, JEOL-JEM-2100F).

2.7.3. Characterization of Ag-NPs using Fourier-Transform Infra-Red spectra (FT-IR)

The functional biomolecules present in the alga which may be responsible for the silver nanoparticles formation were examined and characterized by using FT-IR Spectrometer (FTIR-8400S, Shimadzu, Japan). The dried silver nanoparticles were scanned at the wavelength (400 to 4000 nm) by compressing with potassium bromide (KBr) powder into thin pellets for obtaining their composition.

2.8. Nematicidal activity on second-stage juvenile mortality

A lab experiment was performed to evaluate the nematicidal effect of either the algal extracts and/or the silver nanoparticles synthesized from the two examined algal species on mortality of J2S of *M. incognita*. Second-stage juveniles were treated with the algal extract or different concentrations from green nanoparticles (AgNPs), (S = 9ml AgNPs + 1 ml nematode suspension, S/2 = 4.5 ml AgNPs + 4.5 ml distilled H2O + 1 ml nematode suspension and S/4 = 2.25 ml AgNPs + 6.75 ml distilled H2O + 1 ml nematode suspension) of each green silver nanoparticles .The bioassay was performed in 10-well cell culture plates, and each treatment was represented by approximately 30 freshly hatched J2S per ml. The plates containing distilled water (9 ml distilled H2O with 1 ml nematode suspension) served as a control, Nemacur® 400 EC (9 ml distilled H2O + 1 ml nematode suspension + 10 µl Nema-cu1qaxzwqldr® 400 EC) was used as reference nematicide and there were five replications with repeated twice, the plates were incubated at 25 ± 2°C, and the mortality of J2s was recorded after 12, 24 and 72 h after treatment, The nematodes were considered dead if they appeared motionless in plane water [42,43]. The calculated mortality % was performed using the following formula according to [44].

$$\textbf{Mortality } \% = \left[\frac{(\textbf{Total number of alive J2S in control} - \textbf{No. of alive J2S in treatment})}{\textit{No. of total alive J2S in control}}\right] x100$$

2.9. Nematicidal Activity of the two macroalgal extracts and their synthesized silver nanoparticles against M. incognita in vivo experiment.

Tomato seedlings of 45 day old, cultivar Alisa (The seeds were obtained from Dept. of Vegetable Sciences, Faculty of Agriculture, Alexandria University, Egypt) was used in this experiment. The seedlings were sown in 20 cm diameter sterilized pots filled with autoclaved (121°C for 1 h) and ventilated sandy: clay soil (1:1 v/v) to investigate the nematicidal activity of the algal extract of the two examined algal species (*C. sinuosa* and *C. mediterranea*) and their effects on tomato growth The experiment was carried out in a greenhouse of the Faculty of Agriculture, Alexandria.

Six treatments were applied in this experiment, with 10 replicates for each treatment. The pots were infested with 2000 J2S and eggs of *M. incognita* [45]. The 1st treatment was inoculated only with *M. incognita* (MI) as a positive control. The 2nd treatment was treated with the nematicide Nemacur® 400 EC (1 ml)/pot after being inoculated with 2000 J2S and eggs of *M. incognita*. The 3rd treatment was inoculated with 2000 J2S and eggs of M. incognita simultaneously with *C. sinuosa* extract (40 ml/ pot),

whereas in the 4th treatment silver nanoparticles (100% conc.) were added instead of the normal extract. The 5th and 6th treatments followed the same procedures but were supplied with *C. mediterranea* extract (40 ml/ pot) and silver nanoparticles (100% conc.), respectively. Pots were watered three times a week with about 300 ml of fresh water.

The pots were arranged in a greenhouse in a randomized-block design. The plants were harvested 60 days after nematode inoculation and fully washed from the surrounding soil. The following parameters: fresh and dry weights of root and shoot systems, numbers of nematode root galls, egg masses, and eggs/egg mass were assessed. Egg masses were stained for about 15 minutes, using phloxine B stain (0.15 g/l tap water), then washed with tap water [41].

2.10. Thin Layer Chromatography (TLC)

Since the methylene chloride extract of *C. sinuosa* exhibited higher activity in the bioassay than that of *C. mediterranea*, this extract was further applied to a plate of silica gel (60–120 mesh) thin layer column chromatographic separation. The thin layer chromatography of purchased pre-coated silica plate was set by selecting a small area of 1.5 cm on the plate, where few drops of different eluents of methylene chloride were added, making a distance between each small area at least of 1 cm. The flow rate of the active material was determined using different eluent systems. The elution of the active mate-rial was made using of the following eluents, considering different degree of solvent polarity. Hexane: methylene chloride (9:1 v/v); hexane: methylene chloride: ethyl acetate (1: 0.5: 0.5 v/v) and hexane: methylene chloride: ethyl acetate (2.5: 1: 0.5 v/v). In each case of the chromatograms, the solvent front was marked, spots were identified with pencil, detected un-der a U.V lamp (CAMAG Model, short wavelength 254 λ, high wavelength 365λ), and the retention factor (Rf.) was calculated. The migrating spots of the detected active material were visualized by using UV lamp (UVS-II).

2.11. Preparative thin layer chromatography

A preparative thin layer chromatography was applied to a plate of silica gel (60–120 mesh), to make fractionation of the most effective eluent of methylene chloride; Hexane: methylene chloride: ethyl acetate (1: 0.5: 0.5 v/v).

2.12. GC-MS analysis of methylene chloride crude extract

The most effective methylene chloride eluent fraction (Hexane: methylene chloride: ethyl acetate; 1.5: 0.5: 0.5 v/v) of *C. sinuosa* was further analyses using Gas Chromatography-Mass Spectrometry (GC-MS) and the chemical constituents were detected [46,47]. The analyses were performed in Agilent 7693 series GC method equipped with an OV-5 capillary column (length 30 m x diameter 0.25 mm x film thickness 0.25 µm, Ohio Valley Specialty Chemical, Inc) and an Agilent 5975C network selective mass detector. The extract was arranged by soaking the dry algal material in the eluent 3 consecutive soakings (1:10 w/v)

and the filtrate was exposed to GC-MS analysis (Perkin Elmer), with primary temperature 90^{o}C for 1 min, reaching to 300°C for 30 min, the split less mode with injection volume 1 µl total run time 61.87 min. The mass spectrometer was conducted in the electron impact (El) mode at 70 eV in the scan range of 60–600 m/z. The chemical constituents of the methylene chloride eluent fraction were discovered by comparing the GC-MS peaks with retention times of standards, and the mass spectra obtained were associated with those available in the Mass Spectral Library NIST 2015 [48]. The percentage of each component was estimated as the ratio of the peak area to the total chromatographic area[49].

2.13. Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA) and differences among the means were determined for significance at $p \leq .05$, using revised LSD test using the statically analysis system SAS [50].

3. Results

3.1. Characterization of nanosilver

In (Fig. 1), the SEM image showed the morphologies of the synthesized silver nanoparticles at magnification of 5000X. The nanoparticles were predominantly in the spherical form and some of them were in the form of ag-glomerates (Fig. 1) which were aggregated in anisotropic Ag particles less than 40 nm in size, TEM (Fig. 2). The Fourier-Transform Infra-Red spectra (FT-IR) was used to characterize the biomolecules of the silver nanoparti-cles AgNPs. The spectra showed the presence of prominent and the distinct peaks: 3915,3900,3751, 3421, 2928, 1637,1533, 1386,1327,1228, 1072 and 532 and 474 cm − 1 (Fig. 3).

3.2. Evaluation of C. sinuosa and C. mediterranea extracts and Their SNPs as nematicidal activity of M. incognita Invitro (Lab experiment)

In vitro study of the nematicidal activity of the C. *sinuosa and C. mediterranea* extracts and their syntheses of nanoparticles were tested against the J2S of the root-knot nematode *M. incognita* with concentration (S = 100%, S/2 = 50%, S/3 = 75%, and S/4 = 25%) were compared with Nemacure (commercial nematicide) after 12, 24, and 72h exposure time (Table 1).

Table 1

The effects of *Colpomenia sinuosa*, *Corallina mediterranea* macroalgal extracts and synthesized silver nanoparticles on J2s mortality % (M) of Meloidogyne incognita (MI) after 12, 24 and 72 h of exposure.

Treatment	Exposure time, number of alive J2S (L) and mortality % (M)						
	Con	12 h		24 h		72 h	
L	M	L	M	L	M		
(MI) (control)	-	12.2 a	-	17a	-	29a	-
MI + Nemacur®	-	1.2 d	90.16	0.3f	98.24	0.3g	98.97
MI + *C. sinuosa* extract	S	8.7 b	28.69	4.2de	75.29	9.0c	68.97
MI + *C. sinuosa* SNPs	S	1.6 d	86.89	0.9f	98.24	0.5g	98.28
	S/2	2.4 d	80.33	1.5f	91.18	1.06g	96.34
	S/4	4.9 c	59.84	4.9dc	71.18	3.0f	89.66
MI + *C. mediterranea* extract	S	11.0 a	9.84	6.3c	62.94	13.6b	53.10
MI + *C. mediterranea* SNPs	S	5.9 c	51.64	3.4e	80.00	3.7ef	87.24
	S/2	6.1 c	50.00	4.5de	76.47	5.0e	82.75
	S/4	9.0 b	26.23	8.5b	50.00	6.8d	76.55

Data are means of 5 replicates. Means with the same letter(s), in each column, are not significantly different at $P \leq 0.05$., L = Live. Mortality % =M= [(Total number of a live J2S in control - No. of alive J2S in treatment) / No. of Total alive J2S in control] × 100.

The data indicated that the treatments of MI+ *C. sinuosa* were more effective than that of *C. mediterranea*, increasing J2S mortality of M. incognita with 75.29 and 63% after 24 h, 68.97 and 53 % after 72h exposure time, respectively. On the other hand, the treatment of MI*+ C. sinuosa* NPs with concentration (S) was more effective than the other treatments at all concentrations with 87, 98.24 and 98.28 % after 12, 24, and 72h exposure time, respectively. Which was comparable to the treatment of Nemacur® as control and which was confirmed statistically.

3.3. Evaluation of C. sinuosa and C. mediterranea extracts and Their SNPs as nematicidal activity of M. incognita in vivo (Pot experiment)

The data of Table 2 showed the effect of *C. sinuosa and C. mediterranea* extracts and SNPs compared with Nemacur® 400 EC on the numbers of nematode galls (G), Egg Masses (EM) and number of eggs/egg-masses (E/EM) per tomato plants roots infected with M. incognita (MI) after 60 days. The results revealed that the effect of *C. sinuosa* extract for exceeded that of *C. mediterranea*, regarding the

three parameters (G, EM, and E/EM) with reduction percentage 53.24 and 8.65%, 73.24 and 52.11% and 80.40 and 49.44 %, respectively.

Table 2
The effect of *Colpomenia sinuosa* (Cs), *Corallina mediterranea* (Cm) extracts and synthesized silver nanoparticles and Nemacur® 400 EC on numbers of nematode galls (G), egg masses (EM) and eggs/egg mass (Eggs) and Reduction % (R) in tomato crop infected with M. incognita (MI) after 60 day in a pot experiment Second item.

Treatment	Galls	*R%*	Egg Masses	*R%*	Eggs	*R%*
Control (MI)	92.5a ± 20.42	-	53.3 a ± 17.45	-	221.5 a ± 4.65	-
Plant + MI + Nemacur®	3.4f ± 4.18	96.32	4.75 bc ± 1.11	91.08	5.5 f ± 52.93	97.52
Plant + MI + Cs	43.25c ± 24.30	53.24	14.3 bc ± 3.33	73.24	43.33 d ± 3.64	80.40
Plant + MI + Cs + SNPs	5.4 e ± 2.42	94.16	1.75 c ± 0.48	96.71	15.75 e ± 46.24	92.90
Plant + MI + Cm	84.5 b ± 26.92	8.65	25.5b ± 8.91	52.11	111.5 b ± 49.62	49.44
Plant + MI + Cm + SNPs	22.25 d ± 2.72	75.95	11.5 bc ± 6.51	78.40	100.57c ± 48.85	54.60

Data are means of 10 replicates. Values followed by the same letter(s) are not significantly different at $p \leq 0.05$.

On the other hand, the synthesized nanoparticles of *C. sinuosa* and the Nemacur® 400 EC treatments reduced comparably the number of galls, egg-masses, and eggs/egg-masses of M. incognita by percentage 94.16 and 96.32% to 96.71 and 91.08-% and 92.90 and 97.52 % respectively. The results of these two treatments surpassed that of nanoparticles of *C. mediterranea* for the three parameters and that of the normal extracts of both seaweeds, which showed statistically significant differences.

The effects of *C. sinuosa* and *C. mediterranea* macroalgal extracts, silver nanoparticles and Nemacur® 400 EC on growth parameters of tomato plants infected with *M. incognita* (MI) after 60 days of nematode inoculation are shown in Table (3). The results indicated that when applying the silver nanoparticles of *C. sinuosa*, caused a positive effect on plant growth by enhancing the length of both shoot and root by the centimeter and fresh weight of shoot and root by the gram. The root fresh weight increased by 93.94 % which was higher than that of all the other treatments and that of the positive control. On the other hand, all the treatments had comparable effect on the shoot fresh weight and were lower than that of the positive control.

The data of the shoot dry weight clarified that nano silver (AgNPs) prepared from *C. sinuosa* extract showed an increase by 47. 35%, whereas the algal extract showed 52.25% which were both higher than

that of the positive control 16.77% but lower than that of *C. mediterranea* extract 80.00% and AgNPs 86.45%. While all the treatments had the same effect on root dry weight and were comparable to the positive control, except for AgNPs prepared from *C. mediterranea* (Table 3).

The values of the length of plant shoots and roots did not show any significant differences when applying the treatments or the positive control (Table 4). On the other hand, tomato plants infected with MI and treated with C.s.+ SNPs produced fruits and flowers with higher number than that of the other treatments 1.0 and 4.25, respectively, which was confirmed statistically.

Table 3
The effect of C. *sinuosa (Cs), C. mediterranea* (Cm) macroalgal extracts, synthesized silver nanoparticles and Nemacur® 400 EC on some growth parameters of tomato plants infected with *M. incognita* (MI) after 60 days in a pot experiment and Increase % (I).

Treatment	Shoot system				Root system			
	Fresh weight (g)	I	Dry weight (g)	I	Fresh weight (g)	I	Dry weight (g)	I
Control (Healthy)	22.05 c ± 3.71	0	7.75 c ± 0.63	0	7.09d ± 0.82	0	2.52ab ± 0.32	0
Control (MI)	8.03 d ± 0.64	-	1.23 d ± 0.02	-	2.72e ± 0.34	-	0.99b ± 0.07	-
Plant + MI + Nemacur®	27.39 a ± 2.56	24.22	9.05 c ± 0.58	16.8	9.00c ± 0.91	26.94	3.33a ± 0.25	32.14
Plant + MI + C.s	25.89 ab ± 1.43	17.41	11.80 b ± 0.35	52.3	9.75bc ± 0.5	37.52	3.24a ± 0.11	28.57
Plant + MI + C.s + SNPs	25.09 b ± 0.70	13.79	11.42 b ± 0.00	47.4	13.75a ± 2.3	93.94	2.87 a ± 0.05	13.89
Plant + MI + Cm	26.71 ab ± 1.12	21.13	13.95 a ± 0.03	80.0	13.22a ± 1.1	86.46	2.98 a ± 0.06	18.25
Plant + MI + Cm + SNPs	26.58 ab ± 1.25	20.54	14.45 a ± 0.77	86.5	11.23b ± 0.6	58.39	2.20ab ± 0.00	-

Data are means of 10 replicates. Data expressed as mean ± SD. Values followed by the same letter(s) are not significantly different at $p \leq 0.05$.

Table 4
The effect of C. *sinuosa (Cs), C. mediterranea* (Cm) macroalgal extracts, synthesized silver nanoparticles and Nemacur® 400 EC on some growth parameters of tomato plants infected with *M. incognita* (MI) after 60 days in a pot experiment.

Treatment	Length (cm)		Number of Fruit	Number of Flower
	Shoot	Root		
Control (Healthy)	30.25a ± 4.59	13.25a ± 10.3	1a ± 0.408	2.57 ab ± 1.548
Control (MI)	23.72a ± 3.425	9.75 a ± 8.43	0b	0b
Plant + MI + Nemacur®	28.75a ± 4.131	11.75a ± 10.08	0.5 ab ± 0.289	4 a ± 1.414
Plant + MI + Cs	30.5a ± 4.592	12.5 a ± 10.24	0.25 b ± 0.25	2.5 ab ± 1.041
Plant + MI + Cs + SNPs	31a ± 5.452	15.75a ± 11.2	1.0a	4.25 a ± 1.548
Plant + MI + Cm	23.25a ± 3.082	9 ± 9.05a	0.5 ab ± 0.289	1.75 ab ± 0.629
Plant + MI + Cm + SNPs	25.25a ± 4.708	14 ± 8.4a	0b	0b

Data are means of 10 replicates. Data expressed as mean ± SD. Values followed by the same letter(s) are not significantly different at $p \leq 0.05$.

As well as the thin layer chromatography of different *C. sinuosa* methylene chloride eluents revealed that the most effective one was Hexane: methylene chloride: ethyl acetate (1: 0.5: 0.5 v/v) (Fig. 4A). The fractionation of this effective eluent resulted in four fractions (Fig. 4B), which were applied separately on *M. incognita* (MI) after 12, 24 and 72h of exposure to evaluate their effect on the parasite (MI) (Table 5). The third fraction can be considered as the most effective one, where it resulted in 87.5% mortality after 12h, absolute mortality 100% after 24h and 72h exposure which was comparable to the positive control during the three periods.

Table 5
The effects of *C. sinuosa* methylene chloride eluent fractions on J2s mortality % (M) of *Meloidogyne incognita* (MI) after 12, 24 and 72h of exposure.

Treatment	(J2S mortality %)					
	12 h		24 h		72h	
	L	M (%)	L	M (%)	L	M (%)
(Neg. control) (MI)	3.2 a	-	4.2 a	-	5.2 a	-
(Pos. control) MI + Nemacur®	0.2 c	93.75	0.2 bc	95.24	0.00 b	100
MI + The first fraction	0.6 b	81.25	0.6 b	85.71	0.4 b	92.31
MI + The second fraction	0.6 b	81.25	0.2 bc	95.24	0.0 b	100
MI + The third fraction	0.4 bc	87.50	0.0 c	100	0.0 b	100
MI + The fourth fraction	0.6 b	81.25	0.1 c	97.62	0.0 b	100

Data are means of 5 replicates. Values followed by the same letter(s) are not significantly different at $p \leq 0.05$.

The results of GC-MS of the most effective fraction (the third fraction) of methylene chloride eluent (Hexane: methylene chloride: ethyl acetate; 1.5: 0.5: 0.5 v/v) of *C. sinosa* revealed the presence of seven bioactive constituents, with five major compounds (Table 6 & Fig. 5). They are mainly: dibutyl phthalate and its two isomers 11.68, 4.18 and 22.42%, methy methyltetradecanoate 0.76%, palmitic acid 1.34%, 1-propene-1, 2, 3-tricarboxylic acid, tributyl ester and its two isomers 1.16, 1.04 and 1.25% and finally tributyl acetylcitrate and one isomer 15.57 and 40.60% (Table 6 & Fig. 5).

4. Discussion

This study is an attempt for the application of green nanoparticles as a new approach instead of unsafe chemical nematicides [2,4,51,52], which were synthesized from two marine algal species collected from Abu-Qir and Gleem, Alexandria coast, Egypt. As well as the normal algal extracts were evaluated as alternative to the Nemacur®. The NPs were characterized by using SEM and TEM. The representative SEM micrograph (magnified at 5000X) showed that the obtained silver nanoparticles were aggregated in anisotropic Ag particles. In this trend, Pal et al. [53] reported that the Ag particles were aggregated into nanorods with an average edge length above 100 nm.

However, the TEM images showed monodispersed silver nanoparticles with spherical shape which were less than 40 nm in size. The particle size was increased up to 4000 nm. Noticeably, these results confirmed those obtained by SEM and FT-IR. The crystalline nature of the nanoparticles is evidenced by the selected area electron diffraction patterns with circular or rod spots, where the average particle size in the current study was found to be 22.48, 33.94 and 46.07 nm as revealed in the size distribution graph.

Similarly, Devi and Bhimba [54] reported silver nanoparticles prepared with Ulactuca 20–56 nm, whereas Abdellatif et al. [55] recorded lower range of AgNPs prepared with *Turbinaria turbinata* 8–19 nm.

The (FT-IR) analysis spectrum showed sharp absorbance between 440 and 4000 cm-1 for the synthesized nanoparticles, with distinct peaks 3915- 3900-3751, 3421, 2928, 1637 – 1533, 1386-1327-1228, 1072 and 532 – 474. The observed peaks at 3915 cm-1 and 3900 cm-1 corresponded to O-H stretching vibration, referred to alcohol group and the band at 3751 cm-1 indicated to C-H stretching band. The broad spectrum at 3421 cm-1 referred to the strong stretching vibrations of O-H functional group, showing the presence of alcohol and phenol [54]. The broad spectrum at 2928 cm-1 showed the C–H bond stretching vibration of alkyl (-CH 2-) group. The band at 1637 cm-1 in the spectra corresponded to (–NH–C = O), revealing the presence of N–H bended primary amines. The peak at 1533 corresponded to N-O stretching of aromatic nitro compounds. The peak at 1386 cm-1 is due to symmetric carboxylate stretching, whereas the peak at 1327 referred to N-O asymmetric Stretch, revealing the presence of nitro compounds functional Group. The peaks at 1228 cm-1 and 1072cm-1 were assigned to C–O or C–O–C stretching of aromatic ethers. On the other hand, the very weak bands at 474 and 532 cm-1 indicated the occurrence of vibrations of alkyl halides. Thus, FTIR study revealed the multifunction of nanoparticles synthesized from *Colpomenia sinuosa* extract, where the protein, phenols, and other groups present in the aqueous extract of the *C. sinuosa* are responsible for the reduction of Ag + to AgO and the stabilization of the synthesized AgNPs.

The results of bioassay showed that the treatment with the synthesized nanoparticles of *C. sinuosa* was the most effective treatment, comparable to the effect of the commercial Nemacur® 400 ECe, in eliminating the juvenile of *M. incognita* after 72h exposure with full concentration which decrease with lower concentrations. These results extended to the reduction percentage of the three parameters (the number of galls, egg masses and egg/egg mass) of *M. incognita*. In fact, the silver nanoparticles (AgNPs) synthesized from algae, particularly *C. sinuosa* demonstrated its beneficial effect in root-knot nematode management, which was equal to the effect of Nemacur® 400 EC. On the other hand, the algal extract only was not effective as much as synthesized silver nanoparticles and its nematicidal activities were lower than that of Nemacur® 400 EC. The use of chemical nematicides is usually more effective than other strategies but on the other hand, they have caused significant environmental problems due to their toxic residues and associated environmental damage that resulted in severe restrictions on their use [56]. Algae were considered as good alternative for nematode control [57]. On the other hand, the use of nanoparticles such as nano silver has been adopted in the last few years to control plant pathogens including nematodes [30,58].

Our results agreed with Abdellatif et al. [55], who evaluated the nematicidal effect in greenhouse on eggplants (*Solanum melongena* cv. Login), using synthesized silver nanoparticles incorporated in the algal extract solution prepared from *Ulva lactuca and Turbinaria turbinata.* They found that silver nanoparticles (NPs) in concentration of (12.75 mg.100 ml-1) from both algal species were effective on controlling the root-knot nematode like chemical control in eggplants, which at the same time did not

cause any phytotoxicity in eggplants. Abdellatif et al. [55]attributed the highest nanoparticles beneficial effect to their protection of the plant from additional weakness and stress by their incorporation on the algal extracts, which contain all major and minor nutrients and many organic compounds such as auxins, gibbrellins, and precursor of ethylene and betaine [59]. Abdellatif et al. [55] interpret-ed the mode of action of PNs as not specific and are associated with disrupting multiple cellular mechanisms including membrane permeability, ATP synthesis and response to oxidative stress in cells, [60], they stressed on that combining NPs with algae that supplement the NPs nematicidal effect, results in improving of NPs effective-ness by addition natural compound compared with using algal extract alone. It is worth noting that the allowable boundary of the elemental silver was (2.5 µg/ml) in the drinking water during short-term individual consumption for 1–9 days recommended by the United States Environmental Protection Agency [61]. Consequently, the concentration of Ag in AgNO3 NPs applied in our study to assess the nematicidal properties was lower than this dose (1.08–1.35 µg/ml). However, lipid-soluble extracts from marine macroalgae have been investigated from many times as a source of substances with pharmacological properties. Several different organic solvents have been used to screen algae for antibacterial and nematicidal activity [29,31,55,62]. In this study, methylene chloride was used for extracting the bioactive compounds from two tested macroalgal species, where the algal extract of *C. sinuosa* gave higher anti-nematodes activity against *M. incognita* than that of *C. mediterranea*. Among the potent organic compounds in the present study, are five major compounds: dibutyl phthalate and its two iso-mers, methyl 12-methyltetradecanoate, palmitic acid, 1-propene-1, 2, 3-tricarboxylic acid, tributyl ester and its two isomers and finally tributyl acetylcitrate and its one isomer. In addition to isophorone diisocyanate and 2-Methylenecholestan-3-ol, which were detected by the GC-MS of the most effective fraction, resulting from the preparative thin layer chromatography of methylene chloride eluent (Hexane: methylene chloride: ethyl acetate; 1.5: 0.5: 0.5 v/v) of *C. sinuosa* extract, one of these natural bioactive compounds was found to be from the fatty acids group and others from esters, tetracarboxylic acids and phthalate derivatives. Rizvi et al. [63] reported that iso-phorone diisocyanate possess antibacterial activities, while Shareef et al. [66] recorded that 2-methylene choles-tan-3-ol possess cytotoxic activities. It was reported that palmitic acid exhibit antioxidant, nematicide, pesticide, antifouling, antibacterial, anti-inflammatory, and antifungal activity [64]. Methyl 12-methyltetradecanoate was reported to possess nematicide activity [64]. The bioactive compound 1-propene-1, 2, 3-tricarboxylic acid, tributyl ester is known as aconitic acid. Trans-aconitic acid (TAA) is an isomer of Cis-aconitic acid (CAA). Cuiying et al. [65] discovered that (TAA) showed activity against the plant-parasitic nematode *M. incognita*, while CAA dis-played a much weaker nematicidal effect. In the current study, *C. sinuosa* can be considered as producer of aconitic acid, which shows nematicidal activity. On the other hand, dibutyl phthalate is used as ectoparasiticide [28]. However, many studies reported the nematicidal activities of phthalate derivatives. El-Deen et al. [29] analyzed the algal ethanolic extract of Ulva fasciata as a promising nematicide by GC-MS, which exhibited the presence of organic component such as bis (2-ethylhexyl) phthalate with 63.75%, followed by diethyl phthalate 18.46%. Khan et al. [31] investigated seaweed biochemical potential in two different solvents viz., water and methanol at ratios of 2.5, 5 and 10%. It is observed that methanol

extract (10%) of *Colpomenia sinuosa* recorded egg hatching 82 ± 2.84 % and larval mortality 91 ± 1.76 % after 72h.

5. Conclusions

The synthesized silver nanoparticles prepared from the brown alga Colpomenia sinuosa exceeded the nematicide activity of the commercial Nemacur® 400 EC and that of the algal extract of the same species. Thus, it can be used for the control of Meloidogyne incognita and can be an alternative for the chemical nematicide. However, further studies on purification and isolation of the potent bioactive compounds are necessary to determine which one is the most effective. Overwhelmingly, the employment of such a technique in root-knot nematode management could improve new trends, safe, eco-friendly, and effective against the root-knot nematodes control program. So, further studies are required to prepare bio fabricated green nanoparticle that is toxic and killing for nematodes and having biodegradation modes of action before recommending it for field application and IPM program against plant-parasitic nematodes on the various crops.

Declarations

Author Contributions:

Conceptualization, Dina S. S. Ibrahim and Ahmed M. Elshehawi; Data curation, Nihal Galal El-Din Shams El-Din; Formal analysis, Rehab Y. Ghareeb and Ahmed Abdel-Megeed; Funding acquisition, Bandar S. Aljuaid; Investigation, Rehab Y. Ghareeb, Nihal Galal El-Din Shams El-Din, Ahmed M. Elshehawi and Nader Abdelsalam; Methodology, Dina S. S. Ibrahim; Project administration, Nihal Galal El-Din Shams El-Din; Resources, Rehab Y. Ghareeb, Nihal Galal El-Din Shams El-Din, Dina S. S. Ibrahim and Ahmed Abdel-Megeed; Software, Nader Abdelsalam; Visualization, Dina S. S. Ibrahim; Writing – original draft, Rehab Y. Ghareeb, Bandar S. Aljuaid, Ahmed M. Elshehawi, Ahmed Abdel-Megeed and Nader Abdelsalam; Writing – review & editing, Dina S. S. Ibrahim, Bandar S. Aljuaid and Nader Abdelsalam.. All authors have read and agreed to the published version of the manuscript

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The data utilized to support the findings of this research are included within the article

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Compliance with Ethical Standards

This research is complied with relevant institutional, national, and international guidelines and legislation.

"This research does not include any studies with human participants or animals performed by any of the authors."

Conflicts of Interest:

"The authors declare no conflict of interest.".

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Table

Due to technical limitations, table 6 is only available as a download in the Supplemental Files section.

Figures

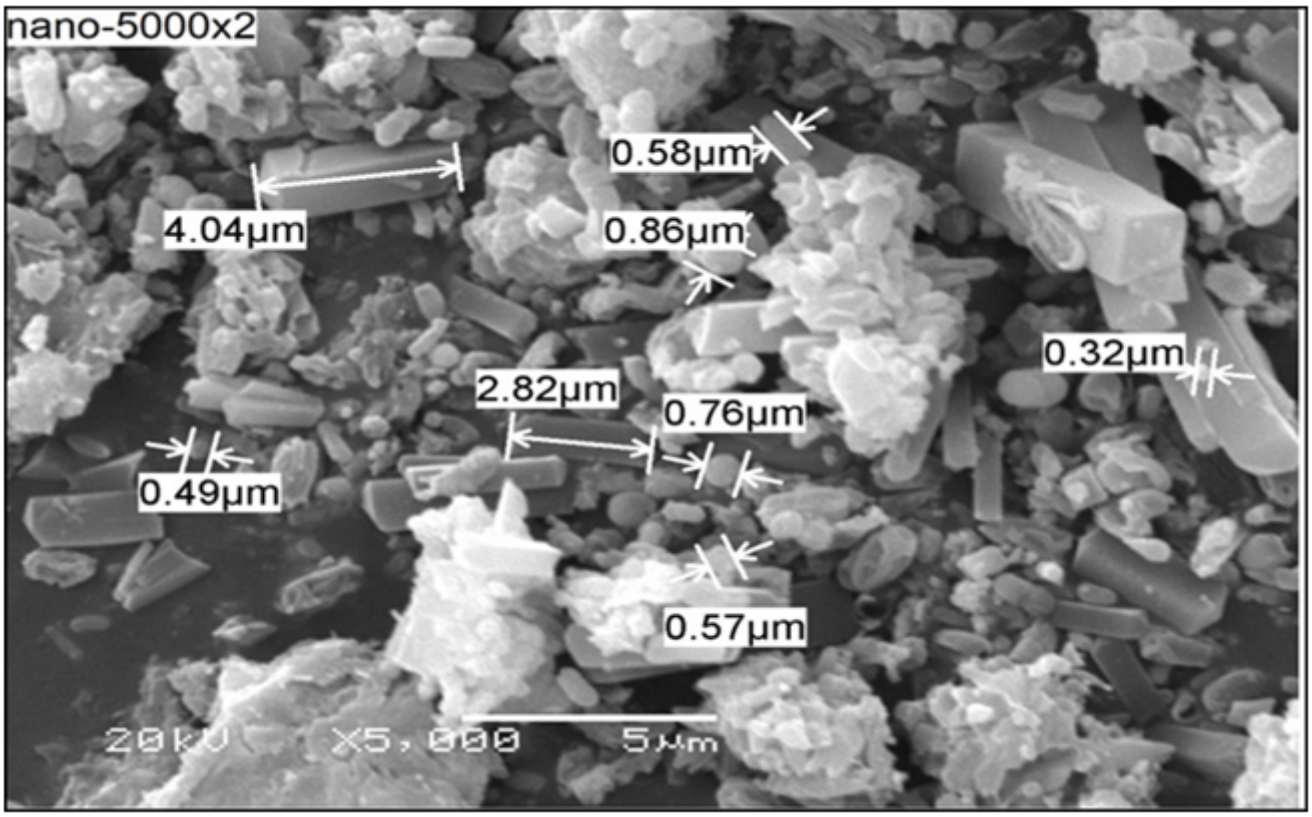

Figure 1

Scanning Electron Microscope (SEM) micrograph of nano silver synthesized by using C. sinu-osa extract magnified at 5000X.

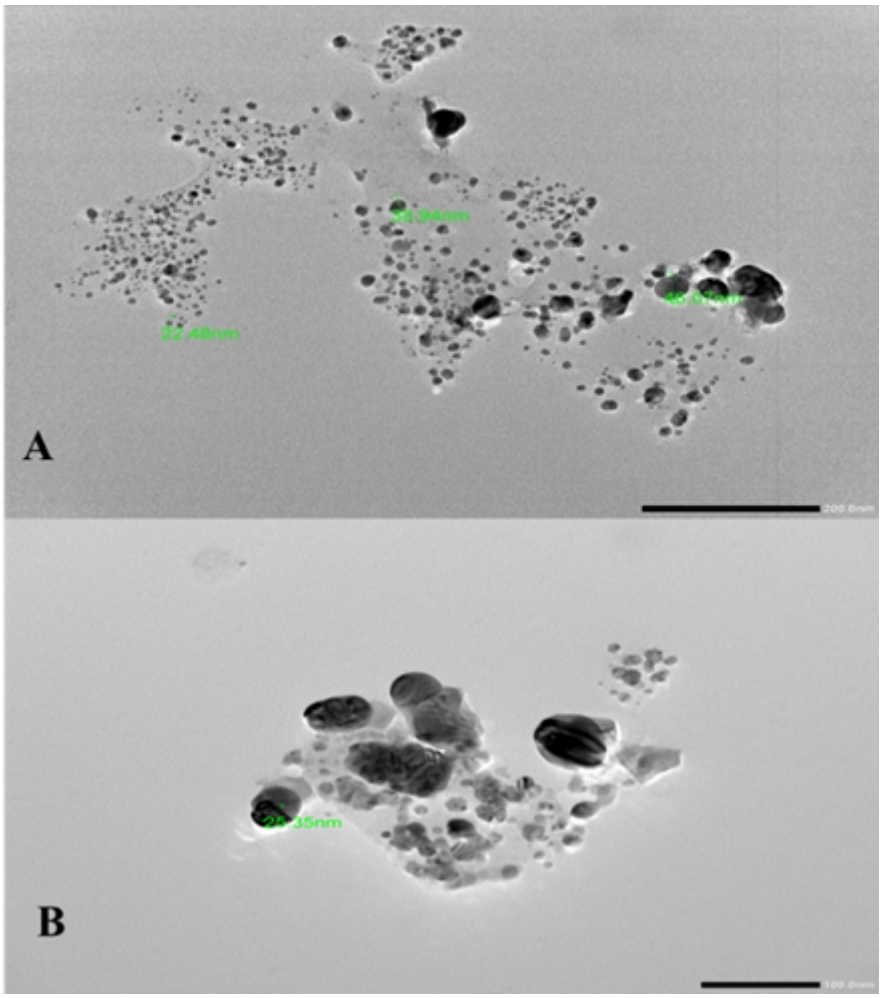

Figure 2

Transmission Electron Microscope (TEM) micrograph of synthesized silver nanoparticles from C. sinuosa magnified at 200X (A) and 100X (B).

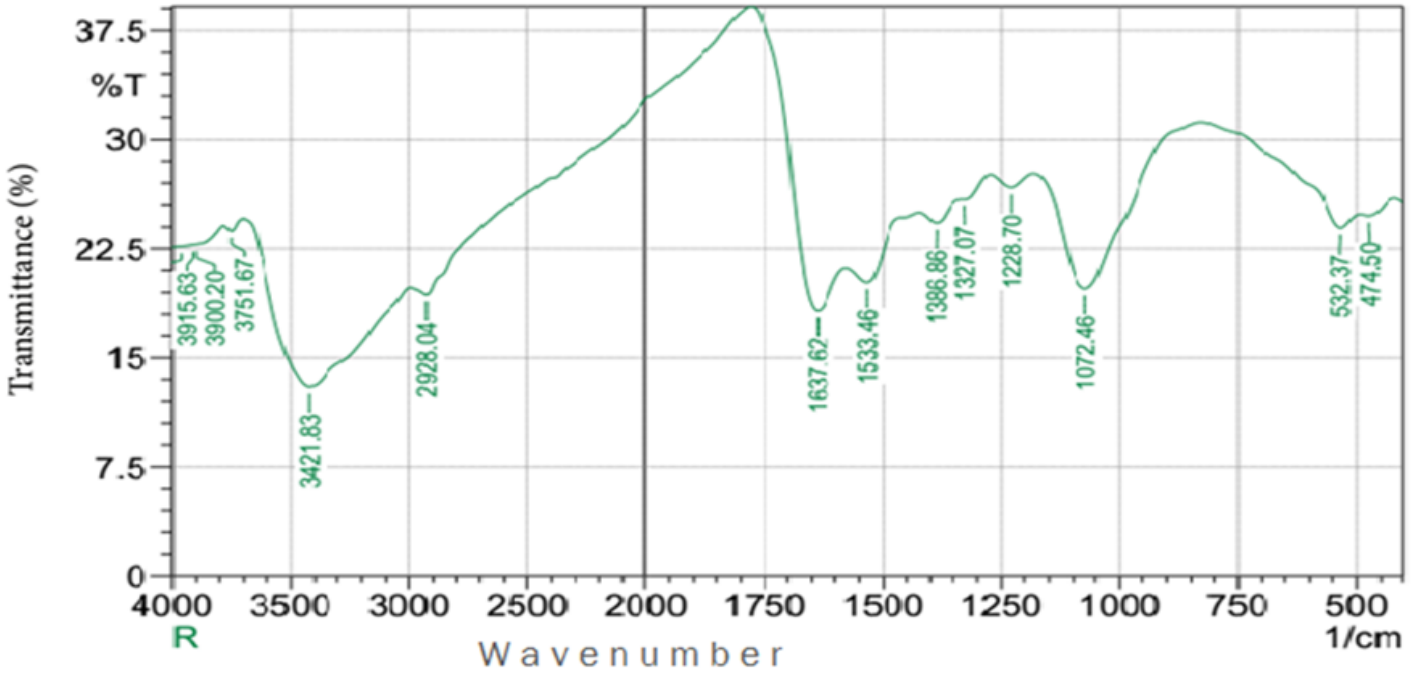

Figure 3

Fourier-Transform Infra-Red spectra (FTIR) spectra shows the functional groups associated with silver nanoparticles synthesized by using C. sinuosa extract.

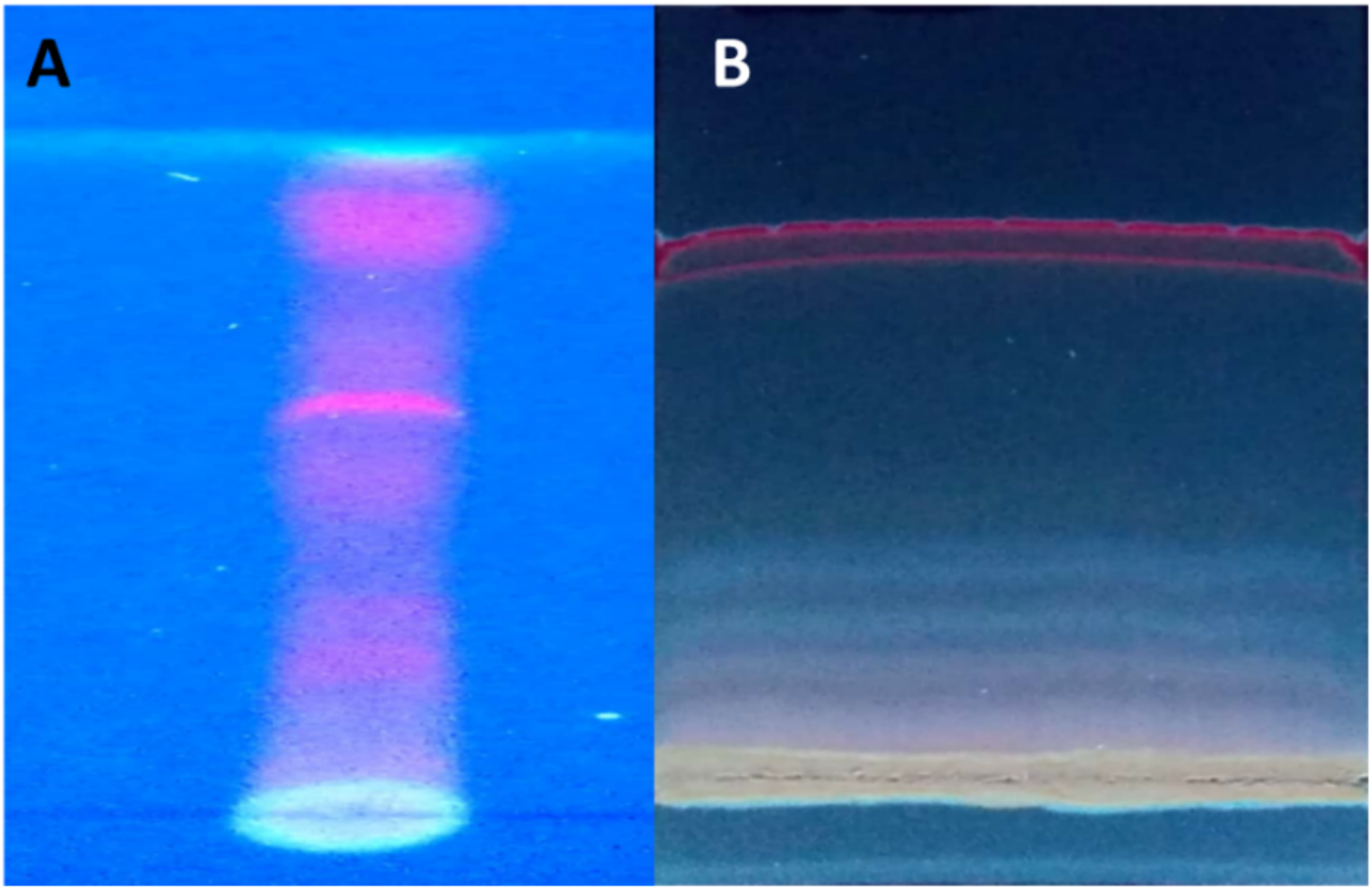

Figure 4

Thin layer chromatography of different C. sinuosa methylene chloride eluents (A) and prepar-ative thin layer chromatography of the most effective eluent (B).

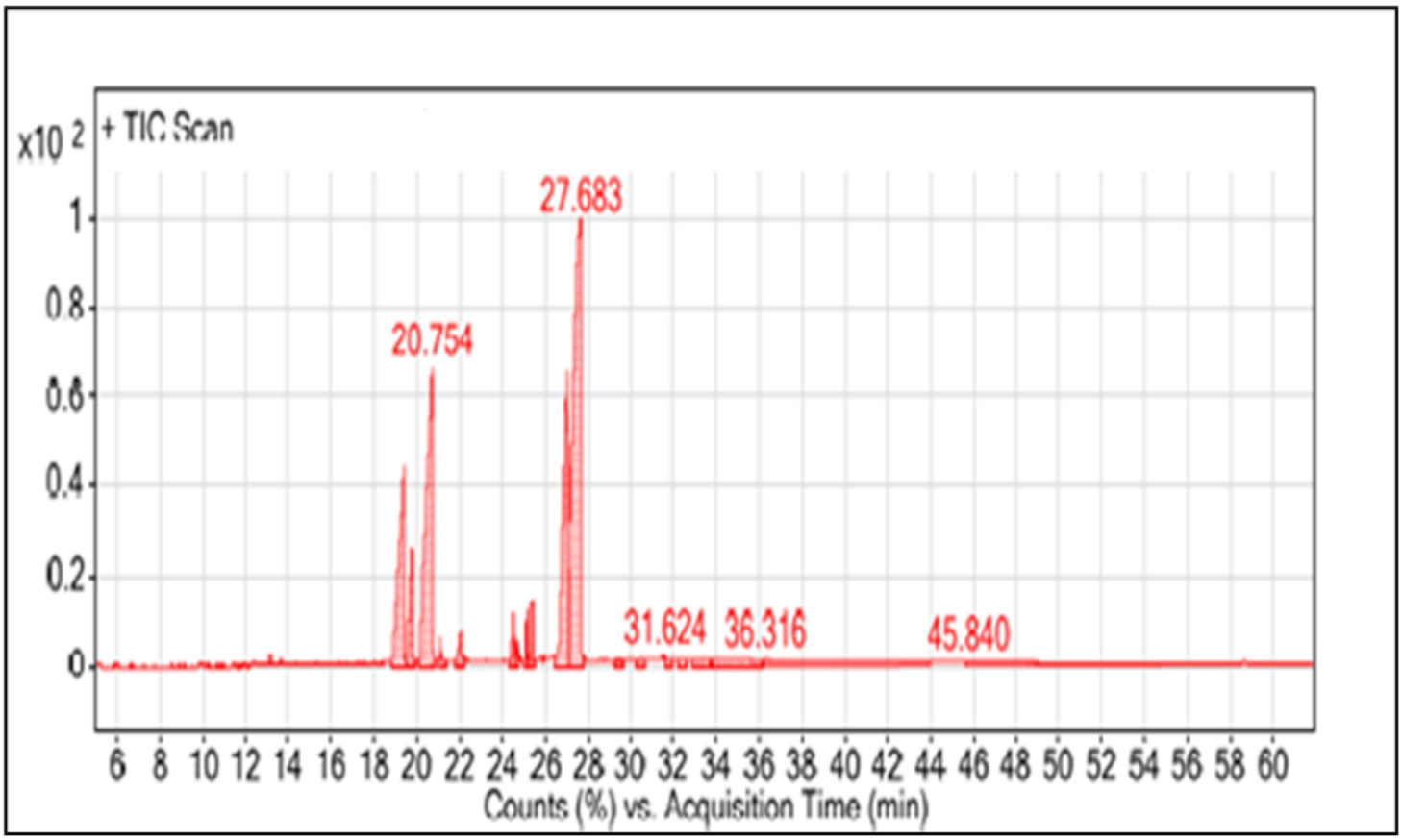

Figure 5

GC-MS chromatograph of the most effective fraction (the third fraction) of C. sinuosa meth-ylene chloride eluent.

Supplementary Files

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- Table6.docx