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Green production of silver nanoparticles, evaluation of their nematicidal activity against *Meloidogyne javanica* and their impact on growth of faba bean

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Abstract

Background: Cyanobacterium-based silver nanoparticles are considered not only as an efficient nano-nematicide but also as a bio-stimulant material for plant growth. They could be employed as a part of an integrated program for controlling some plant diseases.

Results: In this study, silver nanoparticles (Ag-NPs) were biosynthesized from aqueous extract of the cyanobacterium, *Nostoc* sp. PCC7524. Full characterization of the biosynthesized Ag-NPs was monitored by UV-vis spectroscopy, transmission electron microscopy, X-ray diffraction pattern, Zeta sizer, and Fourier transform infrared spectroscopy. In vitro assay against the root-knot nematode *Meloidogyne javanica* showed that Ag-NPs significantly decreased egg hatching of *M. javanica* at different applied concentrations (3, 6, 12, 25, and 50%, v/v). Fifty percent of Ag-NPs induced the highest reduction percent (94.66%). Moreover, Ag-NPs and $AgNO_3$ significantly increased the percentages of larval mortality of the second-stage juveniles (J_2) with concentration and time-dependent responses. Ag-NPs or $AgNO_3$ at 2.4 ml/l, 24 h, completely inhibited the growth of J_2 compared to 23% inhibition using aqueous cyanobacterial extract (ACE). In vivo effect of Ag-NPs on faba bean-infected plant under greenhouse conditions was achieved by treating soil with three different concentrations of 1, 2, and 3 ml/kg soil over two consecutive seasons. Ag-NPs significantly reduced root galling from 39.6 to 78.7% and J_2 population in the soils from 32.2% to 86.7% in the 2018 season and from 21.9 to 78.1% and 40.0 to 81.0% in the 2019 season, respectively. Moreover, 3 ml/kg soil of Ag-NP treatment showed statistically comparable effects to that of vydate nematicide but with remarkable enhancement of faba bean growth parameters as compared to those of vydate or $AgNO_3$ treatments in the two seasons.

Conclusions: The considerable in vitro and in vivo nematicidal potential of the cyanobacterium-based Ag-NPs, besides their bio-stimulant effect on plant growth, makes them feasible for the biological control of *M. javanica*.

Keywords: Silver nanoparticles, Biosynthesis, *Nostoc* sp., *Meloidogyne javanica*, Nematicide, Faba bean

1 Background

The plant-parasitic nematodes especially the root-knot nematode *Meloidogyne* spp. pose a potential threat to most of the agricultural crops in Egypt [1]. Faba bean (*Vicia faba* L.) is a major grain legume widely cultivated in many countries for food and feed purposes [2], and it is one of the oldest legume crops grown in Egypt. The root-knot nematode species attack the roots of the crops and considerably reduce the yield of legumes [3]. Chemical treatment using different types of nematicides has been proposed for the management of root-knot nematodes. However, nematicides do not provide long-term suppression of root-knot nematodes. Furthermore, their environmental and human health problems increased restrictions on their uses [4]. Thus, a considerable number of recent studies extensively focused on the feasibility of biological synthesis of environmentally friendly,

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non-toxic nanocomposites [5–7]. The synthesis of metallic nanoparticles using various biological materials is a green chemistry approach, it is also known as "green biosynthesis." This approach was proposed as an alternative to chemical and physical methods due to few chemicals and less energy input needed for their production.

Indeed, a number of microorganisms including microalgae, fungi, yeast, bacteria [8–11], and plant extract [12] have been used in green biosynthesis of silver nanoparticles (Ag-NPs). Meanwhile, microalgae are considered as a promising tool for nanoparticle production due to their high growth rates and high biomass production. Cyanobacteria, such as *Gloeocapsa* sp., *Lyngbya majuscula*, *Spirulina platensis*, and *Anabaena variabilis*, have been reported for the biosynthesis of intracellular silver, gold, palladium, and platinum nanoparticles (NPs) [7, 13–16]. Qualities of nanoparticles are strongly correlated to their size where the high surface-to-volume ratio of nanoparticles resulted in tremendous increase in their physical, chemical, and biological properties [17].

Several studies reported the use of nanoparticles like Ag-NPs in biological control of different plant-parasitic nematodes [18–20]. However, employing Ag-NPs in the soil is controversial due to their impact on biosystem, e.g., changing the soil bacterial diversity [21], induction of species-specific phytotoxicity in higher plants [22]. Therefore, it is necessary to investigate the mode of interaction of Ag-NPs with plant and their effects on plant growth and development. Previous studies indicated that Ag-NPs induced the variable mode of action on crop plants [23]. In this study, exposure to plant-mediated Ag-NPs resulted in a significant reduction in plant elongation, fresh weight of shoot and root, and total chlorophyll content of seedling of *Lupinus termis* L. Moreover, 0.5 mg/l of Ag-NPs decreased carbohydrates and protein contents and increased accumulation of proline in seedlings [24]. On the contrary, 50 and 75 mg/l of biologically synthesized Ag-NPs protected wheat plants against heat stress and improved plant growth and biomass as compared to control [25]. A similar inductive effect on growth parameters and chemically valuable phytochemical production has been reported for Fenugreek (*Trigonella foenum-graecum* L.) by soaking the seedling in a concentration of 1 μg/ml of Ag-NPs for 5 days [26]. Effectiveness of Ag-NPs is best associated with their chemical composition, size, surface covering, reactivity, and most importantly the applied concentration at which they are effective [27]. The optimum growth promotion and increased root nodulation were observed at 50 mg/l treatment in cowpea (*Vigna sinensis*, var. Pusa Komal), while improved shoot parameters were recorded at 75 mg/l in Brassica (*Brassica juncea*, var. Pusa Jai Kisan) [21]. Therefore, looking for a cost-effective, safe, and efficient nematicide is a serious requirement to ensure quality of life for future generations. The main objectives of the present study were to prepare Ag-NPs using filamentous cyanobacterium *Nostoc* sp. PCC7524 as a green route and to evaluate the nematicidal activity of Ag-NPs against the root-knot nematode *Meloidogyne javanica* infecting faba bean plant in vivo and investigate their impact on plant growth parameters in two consecutive seasons of cultivation.

2 Methods

2.1 Cyanobacterial species and culture condition

The cyanobacterium, *Nostoc* sp. PCC7524 was isolated from the Egyptian agricultural lands and was identified based on the morphotaxonomic and molecular standpoints [28]. The cyanobacterium strain, *Nostoc* sp. PCC7524 was cultivated and propagated for 21 days in 500 ml BG11 medium [29] in 1-L round bottles at 30 °C and under continuous light illumination provided by white fluorescent lamps with light intensity of 30 photon/m^2/s^1. The cultures were aerated with a bubbling air at a regular pressure (200 ml/min and 50 Hz frequency) and sealed with a rubber plug having a narrow glass tube as an air outlet.

2.2 Preparation of cyanobacterial extract and biosynthesis of Ag-NPs

The batch cultures were cultivated until reaching the late exponential growth phase, and the biomass was collected by centrifugation at 5000 rpm for 20 min using a Hettich centrifuge (D-78532 Tuttlingen, Germany). The supernatant was discarded and the pellets were then washed well using deionized water to remove trace salts of the applied growth medium. The fresh biomass was air-dried in clean glass plates and then crushed by a porcelain mortar. The aqueous extract was prepared by soaking 1 g of the cyanobacterial powder in 100 ml of sterilized and deionized water in a 250-ml conical flask. The mixture was shaken thoroughly using a shaker incubator at 200 rpm for 12 h at room temperature. The aqueous cyanobacterial extract (ACE) was obtained by filtration and used as a stock for biosynthesis of Ag-NPs. The biosynthesis was conducted according to the method of Morsy et al. [30] with a little modification (crude ACE was used instead of water-soluble extracellular polysaccharides/matrix; low concentration of silver nitrate ($AgNO_3$) solution was used for biosynthesis). A sample of 50 ml from ACE was mixed thoroughly with 50 ml of 1 mM sterilized $AgNO_3$ in a 250-ml conical flask. Flasks of reaction mixtures were plugged by a cotton stopper and autoclaved for 5 min at 15 psi and 121 °C. The positive control was prepared by mixing 50 ml of ACE with 50 ml of deionized water while solution of 1 mM sterilized $AgNO_3$ was used as a negative control.

2.3 Characterizations of cyanobacterium-based Ag-NPs

Indication of Ag-NP biosynthesis by aqueous extract of *Nostoc* sp. was evidenced by monitoring the UV-visible spectrum using a UV-vis spectrophotometer (UV-2600 Schimadzu, Germany). Samples were measured between 200- and 500-nm wavelengths, and the maximum range was recorded. All measurements were carried out at room temperature. Transmission electron microscopy (TEM) micrographs were obtained by FEI, Netherlands, Tecnai G20 Super twin, double tilt TEM, operating at an accelerating voltage of 200 kV. The sample suspension was sonicated for 5 min to separate the agglomerated particles and to get a homogeneous solution. A drop from the suspension was placed on a film of support grid. The samples were examined after the solvent has completely evaporated. The crystallization degree and elemental composition of the developed Ag-NPs were assessed and identified by an X-ray powder diffractometer (202964 Panalytical Empyrean) with CuKá1 radiation; the voltage and the current of the X-ray source were 40 kV and 30 mA, respectively. The sample was drop-coated onto a silica plate by applying many layers of small amounts of the sample on the plate with intermittent drying, this leads to a thick coat of the sample. Surface charge of cyanobacterium-based Ag-NPs was measured using Malvern Zeta sizer instruments (MAL1121994). Fourier transform infrared spectroscopy (FTIR) spectra is commonly used for identifying biomolecules responsible for the bioreduction of Ag ions and capping of the biosynthesized Ag-NPs. The samples of the freeze-dried synthesized nanoparticles were ground with potassium bromide (KBr), and the spectrum was recorded on FTIR spectroscopy Vertex 70.

2.4 Preparation of *Meloidogyne javanica* culture

The inoculum of *M. javanica* was prepared by collecting individual egg masses of distinct root-knot nematode from diseased tomato plants by a special needle, and then they were immersed in sterilized water for 7–10 days. The obtained new healthy second-stage juveniles (J_2) were reared on tomato seedling planted cv. Super strain B. The tomato seedlings were maintained in a greenhouse at the Department of Plant Pathology, Sakha Agricultural Research Station for more than 45 days to prepare eggs and larval suspensions from the infected roots with galls. Eggs of *M. javanica* were extracted from infected tomato using sodium hypochlorite solution according to Hussey and Barker [31]. The re-extracted nematodes from diseased tomato plants were identified by the examination of Perineal patterns of adult females according to Barker [32]. Identification of *M. javanica* was confirmed by polymerase chain reaction (PCR)

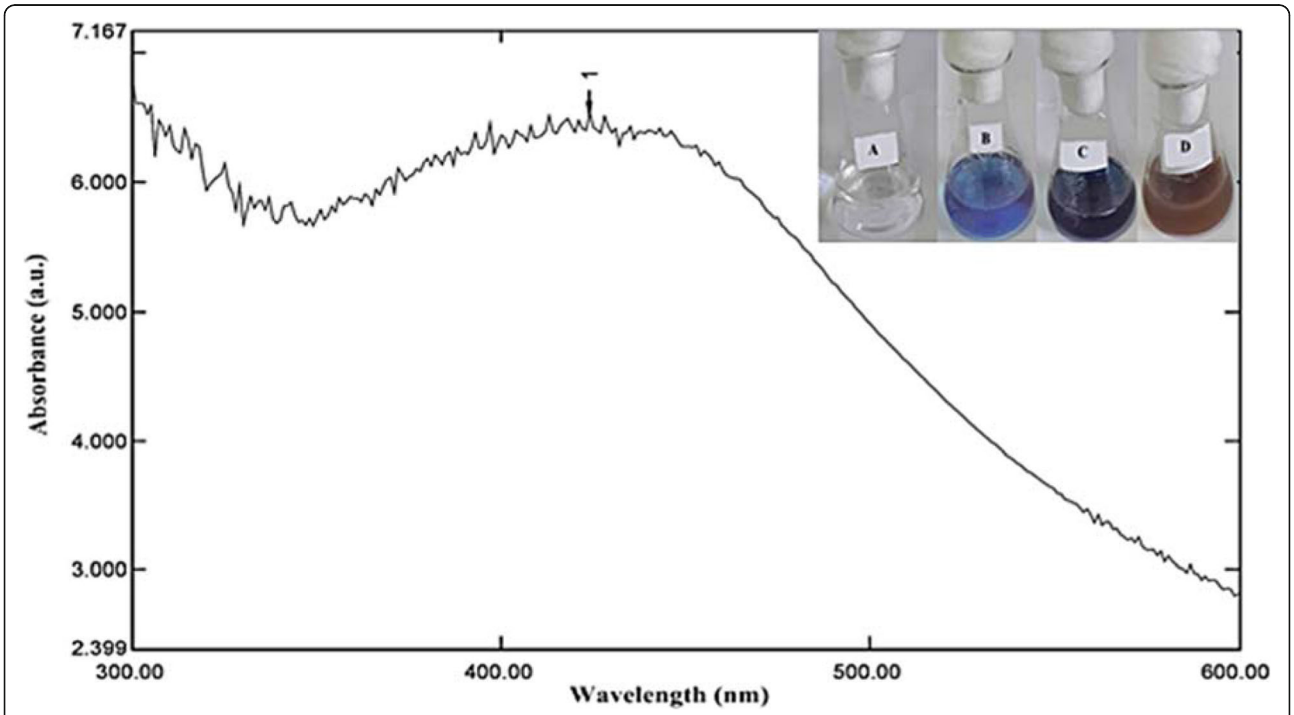

Fig. 1 UV-vis spectroscopy analysis of cyanobacterium-based Ag-NPs using the aqueous extract of *Nostoc* sp. Direct visualization of cyanobacterium-based Ag-NP. (A) Negative control (1 mM $AgNO_3$). (B) Positive control aqueous cyanobacterial extract (ACE). (C) and (D) are ACE with 1 mM $AgNO_3$ before and after heating, respectively

using OPA-01 primer according to a method described by Cenis [33].

2.5 Nematicidal bioassays

2.5.1 Assessment of in vitro nematicidal activity

The inhibitory effect of cyanobacterium-based Ag-NPs, ACE, and $AgNO_3$ solutions against egg hatching of *M. javanica* was performed using five different concentrations (3%, 6%, 12%, 25%, and 50%, v/v). Solutions were prepared by mixing original stocks with sterilized distilled water as diluents. Nine milliliters from each concentration was mixed with 1 ml of egg suspension containing 2000–2500 eggs of *M. javanica* in 25-ml vials. The vials were incubated at 27 °C for 14 days. The percent of egg hatching was recorded using the research microscope. The nematicidal activity of cyanobacterium-based Ag-NPs, ACE, and $AgNO_3$ solutions against the second larval instars (J_2) of *M. javanica* was also managed in vitro by testing five different concentrations (0.15, 0.3, 0.6, 1.2, and 2.4 ml/l, v/v) from the original stocks as aforementioned. Similarly, 9 ml from each concentration was pipetted in 25-ml vials containing 1 ml of juvenile (J_2) suspension (200–300 juveniles). The vials were incubated at 27 °C for 24, 48, and 72 h. The number of active and inactive J_2 larvae was counted, and the percentages of inactive larvae were calculated to evaluate the net percentage (%) of larval mortality at each incubation period. The initial % of inactive J_2 at control conditions was 2.3% after 24 h of incubation, this value was considered in the calculation. Distilled water was used as a negative control. All treatments were conducted in three biological replicates.

2.5.2 Assessment of in vivo nematicidal activity

The nematicidal efficacy of cyanobacterium-based Ag-NPs, ACE, and $AgNO_3$ against *M. javanica* infecting faba bean was performed under greenhouse experiments in two consecutive cultivation seasons (2018 and 2019). Faba bean seeds (Sakha 2 cultivar) were sown in 30-cm-diameter pots filled with a mixture of clay and sand soil in a ratio of 2:1. Two weeks after sowing, the plants were thinned into two seedlings per pot. Each pot was inoculated with approximately 5000 eggs and newly hatching J_2 of *M. javanica* per plant. The inoculums were added inside three holes in the soil, around the base of stems, then pots were irrigated. The inhibitory activity of cyanobacterium-based Ag-NPs, ACE, and $AgNO_3$ was investigated at three different concentrations from each solution (1, 2, and 3 ml/kg soil). Vydate® SL. (24%) was used as the standard chemical nematicide in a dose of 0.01 ml per pot. Treatments were added to pots after infesting the pots with nematode. Infested pots with only *M. javanica* were used as control[1] while untreated pots were used as control[2]. All pots were arranged in the greenhouse in a randomized block design and were kept at 20 ± 5 °C. After 60 days, faba bean plants were uprooted, and the roots were gently washed with a stream of distilled water. The roots with nematode galls were rated on a 1–9 scale of gall index (GI) according to Sharma et al. [34] as follows: 1 = no galls, 2 = 1 to 5 galls, 3 = 6 to 10 galls, 4 = 11 to 20 galls, 5 = 21 to 30 galls, 6 = 31 to 50 galls, 7 = 51 to 70 galls, 8 = 71 to 100 galls, and 9 = > 100 galls per root system. The number of galls developed on roots, final nematodal population (J_2) in 250 cm^3 of soil, fresh and dry weights of shoots, length of plants, and number of flowers per plant was determined. All treatments were conducted in three biological replicates.

2.6 Statistical analysis

Analysis of variance (ANOVA) and regression coefficient analyses were performed by WASP-Web Agri Stat

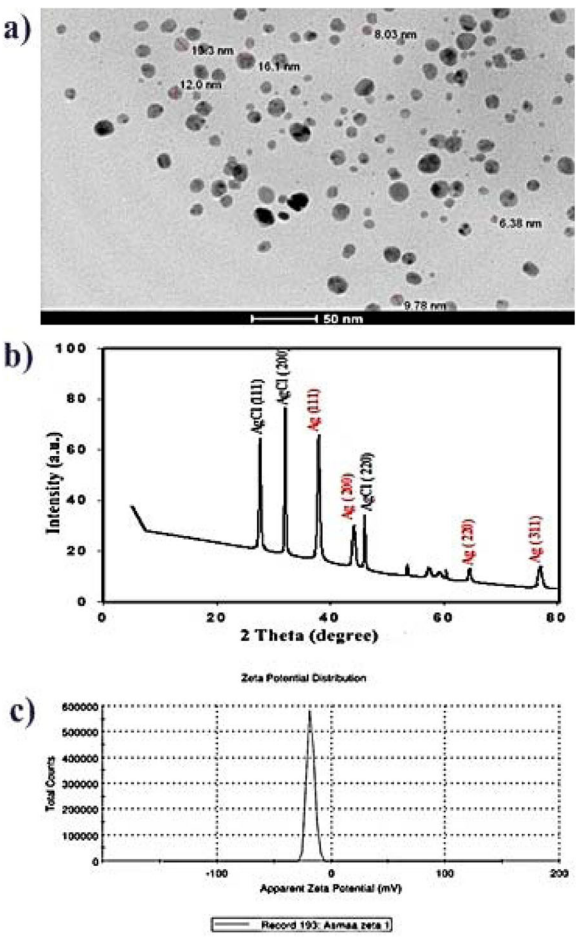

Fig. 2 Characterization of cyanobacterium-based Ag-NPs by reduction of 1 mM Ag^+ ions by the aqueous extract of *Nostoc* sp. **a** Transmission electron microscopy (TEM) micrograph. **b** X-ray diffraction pattern of purified Ag-NPs. **c** Zeta potential analysis of Ag-NPs

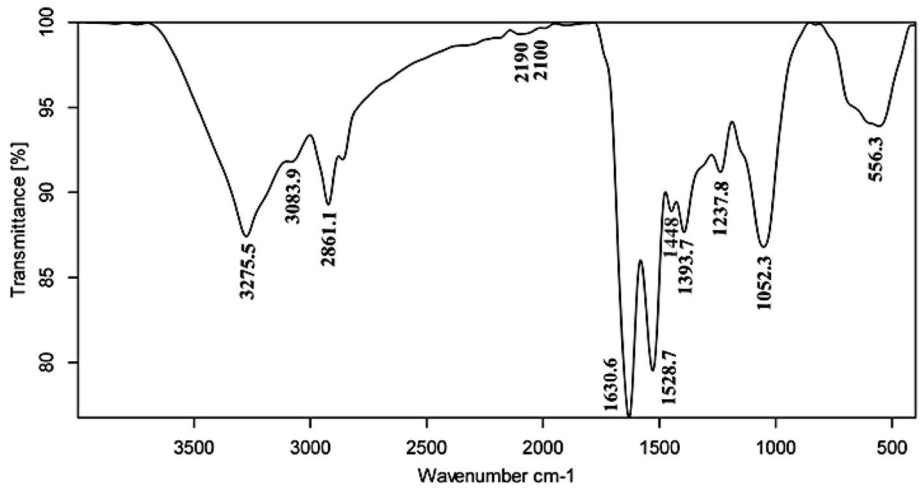

Fig. 3 FTIR spectrum of cyanobacterium-based Ag-NPs bioformed by reduction of 1 mM Ag^+ ions by the aqueous extract of *Nostoc* sp

Package statistical analysis software. Treatment means were separated using Duncan's multiple range test [35]. All analyses were conducted at the significance value of $p \leq 0.05$.

3 Result

3.1 Characterization of cyanobacterium-based Ag-NPs

A distinct shifting in color from light-blue (Fig. 1c) to brown (Fig. 1d) was observed in reaction mixture flask of aqueous cyanobacterial extract (ACE) with 1 mM $AgNO_3$ after 5 min of heating. The obtained UV-vis spectroscopy spectrum showed a single surface plasmon resonance (SPR) absorption band at 424 nm (Fig. 1). TEM micrographic analysis revealed the formation of predominantly spherical structures with an average size which ranged between 6.4 and 16 nm (Fig. 2a). The crystallinity of the cyanobacterium-based Ag-NPs was also confirmed by the X-ray diffraction pattern (XRD) (Fig. 2b). The diffraction pattern revealed the existence of 4 distinct peaks at 2θ values of 38.0°, 44.2°, 64.5°, and 76.9° which were assigned to 111, 200, 220, and 311 crystal planes, respectively, of fcc Ag^0 (JCPDS, card file no: 04-003-7118), and other 3 peaks at 2θ values of 27.6°, 32.1°, and 46.1° were indexed as 111, 200, and 220 crystal planes, respectively, of fcc AgCl (JCPDS, card file no: 04-007-3906). The intensity of the peaks denotes to intensity of crystallinity of the produced Ag and AgCl-NPs. The obtained cyanobacterium-based Ag-NPs were also demonstrated through their characteristic peak of the surface charge observed in Zeta size image, where the bioformed nanoparticles showed an average potential value with − 18.0 mv (Fig. 2c). FTIR spectroscopy analysis was used to identify the possible biomolecules which existed in the aqueous extract of *Nostoc* sp. (Fig. 3). The obtained pattern showed 10 distinct peaks at 3275, 3083, 2861, 1630, 1528, 1448, 1393, 1237, 1052, and 556 cm^{-1}.

3.2 In vitro nematicidal activity of cyanobacterium-based Ag-NPs

Data listed in Table 1 indicate that cyanobacterium-based Ag-NPs, ACE, and $AgNO_3$ remarkably decreased the percentages (%) of egg hatching of *M. javanica* in a concentration-dependent manner. Among tested treatments, cyanobacterium-based Ag-NPs showed a significant

Table 1 Effect of the different concentrations of cyanobacterium-based Ag-NP, ACE, $AgNO_3$ solutions on the percentage of egg hatching of *Meloidogyne javanica*

Treatments	Concentrations (%)	Egg hatching (%)	Reduction (%)
Cyanobacterium-based Ag-NPs	3	17.45fg	72.60
	6	13.38gh	78.99
	12	9.80hi	84.61
	25	5.05i	92.07
	50	3.40i	94.66
Aqueous extract of *Nostoc* sp. (ACE)	3	37.21c	41.58
	6	34.32cd	46.12
	12	24.59ef	61.37
	25	18.61fg	70.78
	50	11.97gh	81.20
$AgNO_3$ solution	3	53.57b	15.90
	6	52.73b	17.22
	12	28.64de	55.03
	25	13.85gh	78.25
	50	9.98hi	84.33
Control (distilled water)		63.70a	0.00

Mean values followed by a common letter(s) are not significantly different at the 5% level by DMRT

reduction in % of egg hatching by 72.6–84.6% ($p \leq 0.05$) at the concentrations 3–12.5%. Additionally, 50% of cyanobacterium-based Ag-NPs and $AgNO_3$ showed the highest inhibitory effect against egg hatching by 94.7% and 84.3% respectively which were statistically comparable ($p \leq 0.05$). Interestingly, low concentrations of cyanobacterium-based Ag-NPs (3% and 6%) showed a significant toxic effect on egg hatching as compared to $AgNO_3$ ($p \leq 0.05$) by 4.56- and 4.58-folds, respectively. Regression analysis in Fig. 4 showed positive and significant relationships between concentrations of cyanobacterium-based Ag-NPs and ACE with reduction % of egg hatching by $R^2 = 0.790$ and 0.871, respectively, whereas a positive insignificant relationship was found between the concentration of $AgNO_3$ and the reductions % of egg hatching by $R^2 = 0.755$ ($p \leq 0.05$).

Percentages of J_2 larval mortality are shown in Table 2. Overall, Ag-NPs and $AgNO_3$ significantly increased % of larval mortality with concentration and time-dependent responses ($p \leq 0.05$). Meanwhile, the cyanobacterial-based Ag-NPs showed insignificant values of larval mortality compared to $AgNO_3$ solution at all tested concentrations. Upon exposure to 2.4 ml/l of cyanobacterial-based Ag-NPs or $AgNO_3$, growth of J_2 was completely inhibited. On the contrary, 2.4 ml/l of ACE showed the lowest percentage of larval mortality by 23–39% at 24–72 h of incubation periods, respectively. The detected LC_{50} values of cyanobacterial-based Ag-NPs were 0.6 ml/l at 24 h and ˂ 0.15 ml/l at 48 h or 72 h of incubation periods, respectively. Results of regression analysis showed positive and significant relationships between different concentrations of the cyanobacterium-based Ag-NPs, ACE, and $AgNO_3$ solutions and the percentage of larvae mortality at different incubation periods with R^2 ranging from 0.745 to 0.973. For instance, cyanobacterial-based Ag-NPs and ACE increased % of larval mortality at all

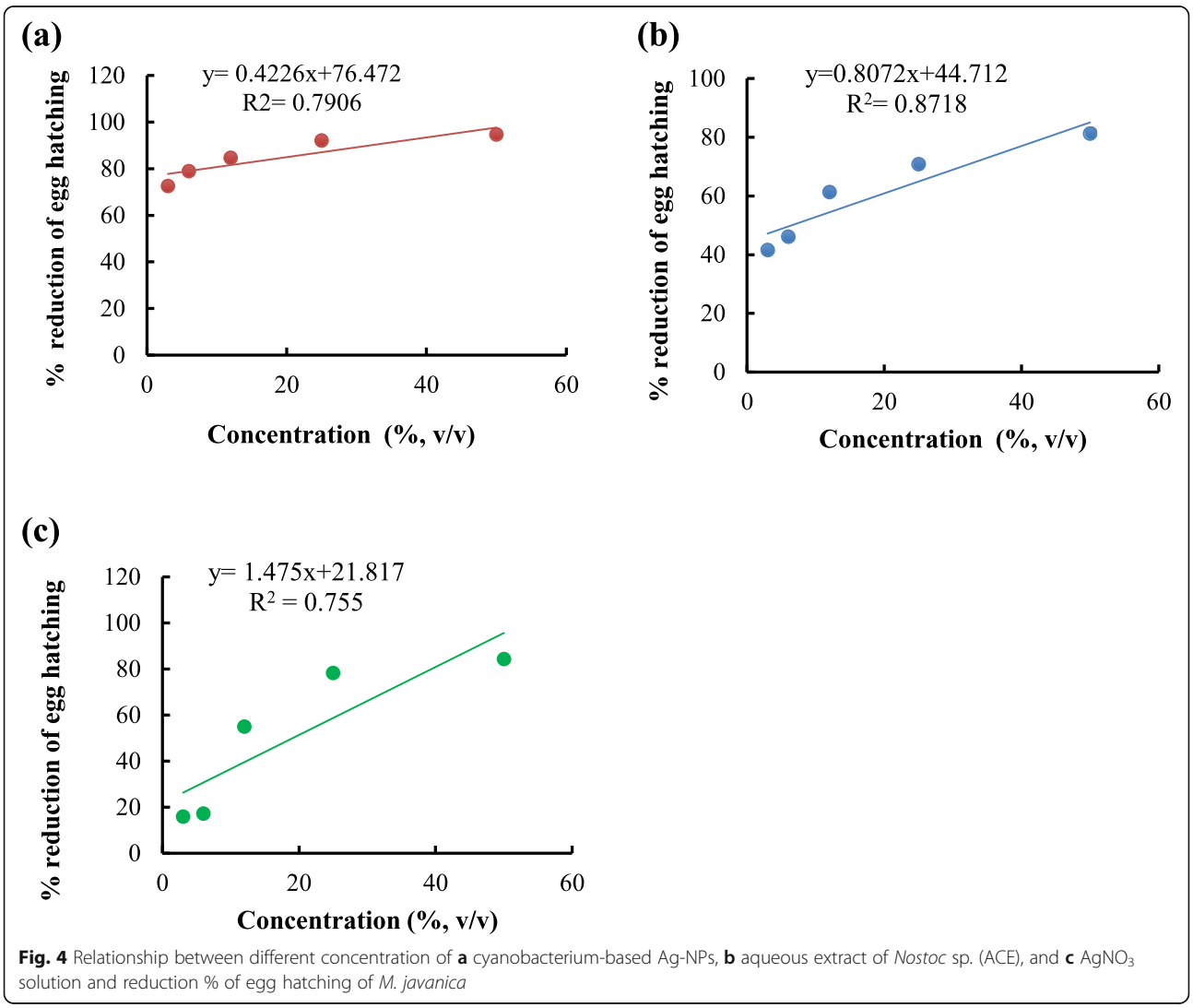

Fig. 4 Relationship between different concentration of **a** cyanobacterium-based Ag-NPs, **b** aqueous extract of *Nostoc* sp. (ACE), and **c** $AgNO_3$ solution and reduction % of egg hatching of *M. javanica*

Table 2 Effect of the different concentrations of the cyanobacterium-based Ag-NP, ACE, $AgNO_3$ solutions on the percentage of larvae mortality of the J_2 juveniles of *M. javanica* at different incubation periods

Treatments	Concentrations (ml/l)	Mortality %		
		24 h	48 h	72 h
Cyanobacterium-based Ag-NPs	0.15	34.55f	55.18de	54.94ef
	0.3	45.73de	65.85cd	63.16d
	0.6	50.20d	62.45cd	86.31b
	1.2	75.00b	82.11b	90.60b
	2.4	100a	100a	100a
Aqueous extract of *Nostoc* sp. (ACE)	0.15	10.40i	12.26hi	15.16i
	0.3	16.10hi	18.13gh	16.10i
	0.6	12.40i	20.30gh	15.26i
	1.2	19.36gh	25.00fg	23.33h
	2.4	23.10g	34.00f	39.33g
$AgNO_3$	0.15	35.07f	48.63e	51.96f
	0.3	40.46ef	60.20cd	60.66de
	0.6	65.56c	60.50cd	80.30c
	1.2	74.89b	82.00b	90.33b
	2.4	100a	100a	100a
Control (distilled water)		2.33j	2.60i	3.13j

Mean values followed by a common letter(s) are not significantly different at the 5% level by DMRT

tested concentrations ($p \leq 0.05$) (Fig. 5a–f). In contrast, $AgNO_3$ treatment showed a significant effect only at high concentrations (˃ 1.2 ml/l) ($p \leq 0.05$) (Fig. 5g–i).

3.3 In vivo nematicidal activity of cyanobacterium-based Ag-NPs on *M. javanica*

In the present study, we observed that soil treated with cyanobacterium-based Ag-NPs or $AgNO_3$ solution showed a similar inhibitory effect on reduction of the root galling and nematode population of *M. javanica* over the two conducted seasons (Table 3). Their inhibitory effects were dose-dependent and were significantly higher than that of ACE ($p \leq 0.05$). For instance, Ag-NPs significantly reduced root galling and J_2 population in the soils from 39.62 to 78.67% and 32.22 to 86.66%, respectively, in the 2018 season and from 21.87 to 78.12% and 40 to 81%, respectively, in the 2019 season as compared to control. Additionally, 3 ml/kg soil of cyanobacterium-based Ag-NPs or $AgNO_3$ solution showed statistically comparable effects on root galling and J_2 population as compared to the vydate nematicide at both seasons. In contrast, ACE showed the lowest reduction value of root galling and J_2 population in the infected soil. Root morphology of faba bean with developed galls of *M. javanica* at the end of the experiment is shown in Fig. 6a–f.

3.4 Influence of cyanobacterium-based Ag-NPs on growth parameters of faba bean

All tested concentrations of cyanobacterium-based Ag-NPs, $AgNO_3$, and ACE significantly improved some growth parameters of faba bean plant but with variable degrees as compared to control[1] (nematode-infected soil) and control[2] (untreated soils) during seasons 2018 and 2019 (Table 4). For more details, upon treating soil with 3 ml/kg soil of cyanobacterium-based Ag-NPS or ACE significantly improved the fresh weight of shoots by 71% and 72% ($p \leq 0.05$), respectively, and increased the dry weight of shoots by 58% and 66% ($p \leq 0.05$), respectively. In addition, both solutions also enhanced the plant heights by 83% and 80% and increased the number of flowers per plant by 206.9% and 248.8%, respectively, as compared to (control[1]) during the 2018 season. Comparable results have been confirmed in 2019 where cyanobacterium-based Ag-NPS and ACE at a concentration of 3 ml/kg soil noticeably increased fresh weight of shoots by 70% and 74%, respectively, and dry weight of shoots by 64% and 81%, respectively. Similarly, plant heights were induced by 59% and 58%, respectively, and the number of flowers/plant were also increased by 103% and 125%, respectively, compared to control[1]. Overall, 2 and 3 ml/kg soil from cyanobacterium-based Ag-NPs and ACE significantly increased the fresh weight of plant shoots ($p \leq 0.05$) as compared to $AgNO_3$, vydate, and control[1,2] during the season of 2018. Although $AgNO_3$ solution showed a significant reduction of root galling and J_2 population in the soil, it had the lowest effect on improving plant growth parameters as compared to cyanobacterium-based Ag-NPS or ACE.

4 Discussion

4.1 Characterization of cyanobacterium-based Ag-NPs

Biosynthesis of cyanobacterium-based Ag-NPs was confirmed by a set of physicochemical analyses. The UV-vis spectroscopy spectrum showed a distinct absorption peak at 424 nm, this might be attributed to the excitation of surface plasmon resonance (SPR) of the synthesized Ag-NPs [36]. Many investigators indicated that Ag-NPs exhibited UV-vis absorption spectra which ranged between 410 and 440 nm which were also assigned to other different metal nanoparticles [6, 7, 37]. The negatively charged polysaccharides of the algal extract tightly bonded with Ag-NPs, and these confined the free electrons of the nanoparticles in a smaller volume, which resulted in a high free electron density and a high plasmon frequency. Therefore, a sharp peak was observed at a lower wavelength [38]. The produced brown color also indicated the green biosynthesis of Ag-NPs and reduction of Ag^+ ions. We assumed that Ag^+ was

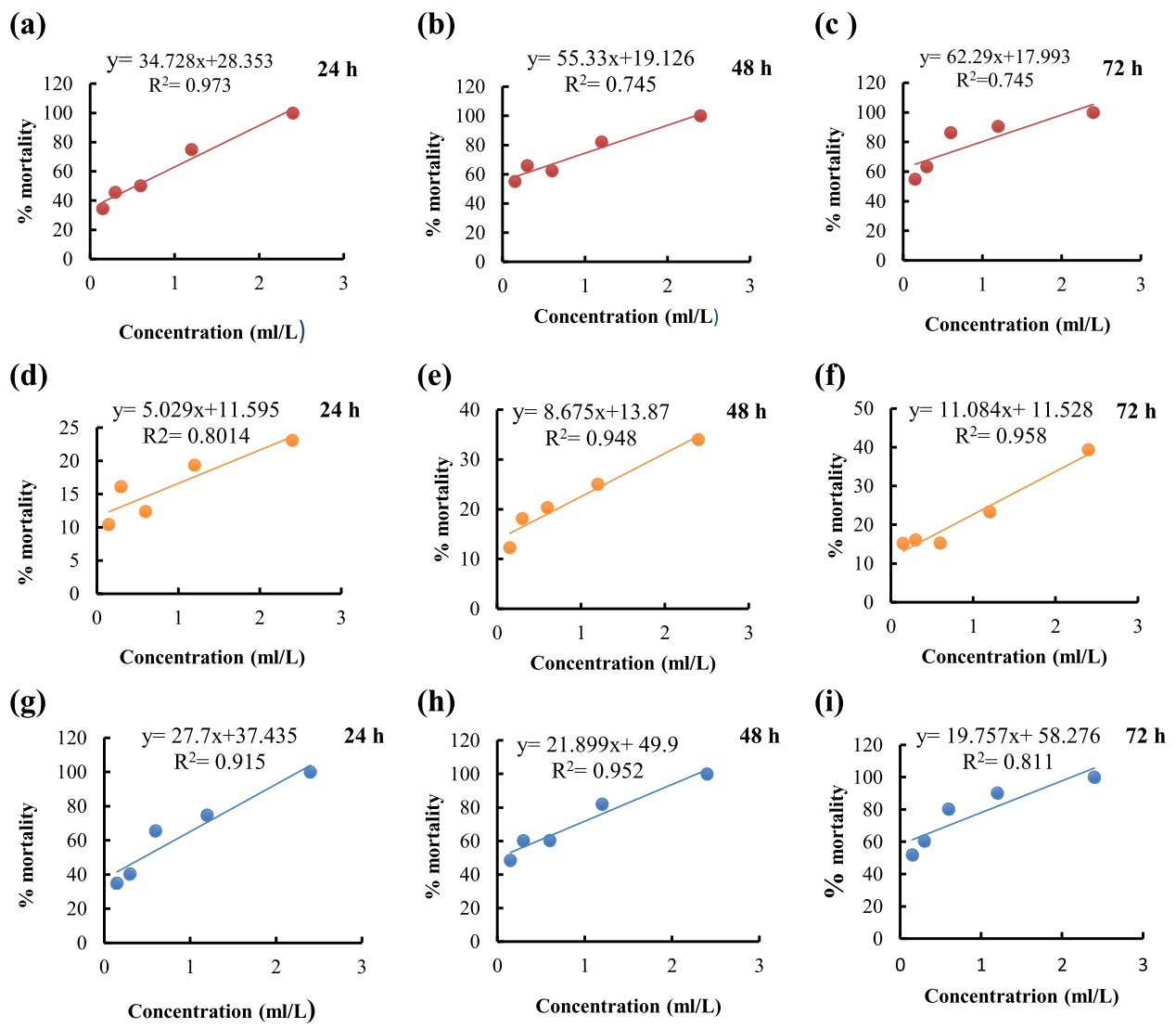

Fig. 5 Relationship between different concentrations of the cyanobacterium-based Ag-NPs (**a**–**c**), different concentrations of aqueous extract of *Nostoc* sp. (**d**–**f**), and different concentrations of $AgNO_3$ solution (**g**–**i**) on the percentage of larvae mortality of the J_2 juveniles of *M. javanica* at different incubation periods

reduced to Ag^0 NPs by some biomolecules that exist in the ACE. It is worth mentioning that chlorine content in the algal extract, previously obtained from BG-11 medium components could be elucidated as the main source for the formation of AgCl-NPs in this study. This observation is in accordance with other previous studies which used bacterial and microalgal species for AgCl-NP biosynthesis [6, 7, 39]. In the present study, Zeta sizer measurement revealed a high potential value of the bioformed Ag-NPs. This characteristic feature might be due to the high negative-negative repulsion force between the nanoparticle which supports well the long-term stability, good colloidal nature, and high dispersity of the Ag and AgCl-NPs [40, 41].

FTIR spectrum showed a broad peak at 3275 cm^{-1} corresponding to primary amide linkage in the protein [42], also 3083 cm^{-1} indicated the presence of stretching vibration modes of secondary amines [43]. The peak at 2861 cm^{-1} was attributed to C–H stretching vibrations of thiols [44]. Moreover, the strong absorption band at 1630 cm^{-1} was characteristic of amide I [45] while the peak at 1528 cm^{-1} indicated the presence of amide II bonds involving carbonyl and N–H stretching of the proteins [46]. The peak located at 1448 cm^{-1} suggested the involvement of CH_2 and CH_3 groups of protein [47]. Similarly, the peak observed at 1393 cm^{-1} was corresponding to asymmetric deformation of CH_3 and CH_2

Table 3 Effect of the different concentrations of the cyanobacterium-based Ag-NP, ACE, $AgNO_3$ solutions and vydate nematicide on the number of galls developed on faba bean roots and J_2 population of *Meloidogyne javanica* in soil under greenhouse conditions during 2018 and 2019 seasons

Treatments	Concentrations (ml/kg soil)	Season 2018					Season 2019				
		Gall index (GI)	Galls/root system		J_2/250 cm^3 of soil		Gall index (GI)	Galls/root system		J_2/250 cm^3 of soil	
			No.	Reduction%	No.	Reduction%		No.	Reduction%	No.	Reduction%
Cyanobacterium-based Ag-NPs	1	7.7abc	80.00cd	39.62	203.33cd	32.22	8.0a	83.33b	21.87	120.00def	40.00
	2	7.0c	63.25de	52.26	151.66e	49.44	6.3bc	53.33c	50.00	93.33f	53.33
	3	5.0d	28.25f	78.67	40.00f	86.66	4.0e	23.33d	78.12	38.00g	81.00
Aqueous extract of *Nostoc* sp. (ACE)	1	9.0a	123.33a	6.92	266.66ab	11.11	8.0a	96.66ab	9.37	180.00ab	10.00
	2	8.2 abc	112.50ab	15.09	230.00bc	23.33	8.0a	90.00ab	15.62	153.33bcd	23.33
	3	8.5ab	103.75abc	21.69	186.66de	37.78	7.6ab	80.00b	24.99	136.66cde	31.67
$AgNO_3$	1	7.5abc	82.50bcd	37.73	216.66cd	27.78	7.8ab	86.66b	18.75	160.00bc	20.00
	2	7.2bc	77.50cd	41.50	153.33e	48.89	6.2cd	56.66c	46.87	100.00ef	50.00
	3	5.3d	31.25ef	76.41	48.33f	83.89	4.6de	26.66d	75.00	50.00g	75.00
Vydate 24% SL.		3.3e	11.50f	91.32	20.00f	93.33	3.4e	11.00d	89.68	32.00g	84.00
Nematode-infected soil (control[1])		8.7a	132.50a	–	300.00a	–	8.3a	106.66a	–	200.00a	–

Mean values followed by a common letter(s) are not significantly different at the 5% level by DMRT

in proteins [48]. The peak located at 1237 cm^{-1} could be assigned to –C–O– of aromatic acids [49] while the peak observed at 1052 cm^{-1} suggested the –C–O stretching vibrations from polysaccharides [50]. Also, the broad absorption band 556 cm^{-1} indicated the possible involvement of alkyl halides [42]. Overall, FTIR data suggested that the proteins and amino acid residues in the cyanobacterial extract that have strong binding ability with metals, possibly formed a layer surrounding the metal nanoparticles and acted as a capping agent to prevent agglomeration of NPs [47]. In addition, polysaccharides perhaps involved in the biosynthesis and capping of Ag-NPs produced by *Nostoc* sp. Our results are in agreement with previous studies of Ali et al. [49] and Ahmed et al. [43] who proposed that proteins were responsible for the reduction and stabilization of silver nanoparticles and metal nanoparticles. Rajeshkumar et al. [45] and Ferreira et al. [37] suggested that proteins

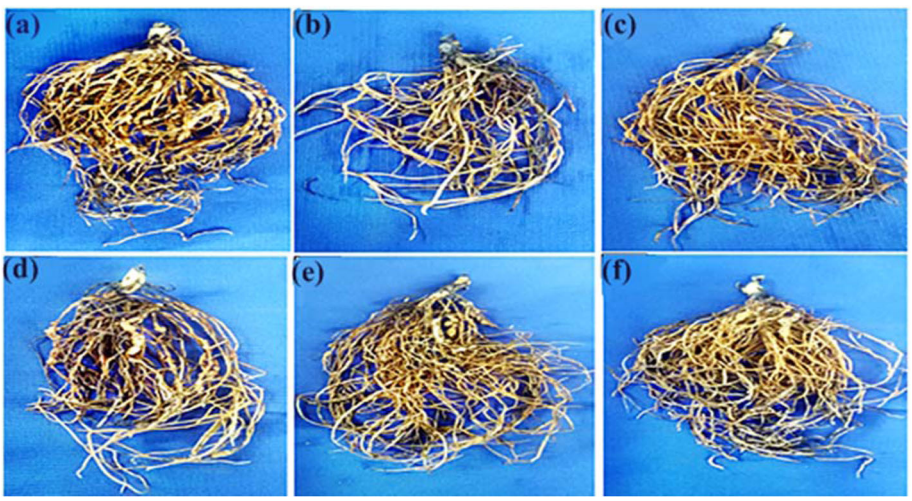

Fig. 6 a–**f** Representative faba bean root galling. **a** (Control[1]) nematode-infected soil. **b** (Control[2]) untreated soil. **c** Treated plants with vydate nematicide. **d** Treated plants with ACE. **e** Treated plants with $AgNO_3$ solution. **f** Treated plants with cyanobacterium-based Ag-NPs

Table 4 Effect of the different concentrations of cyanobacterium-based Ag-NP, ACE, $AgNO_3$ solutions and vydate nematicide on some growth parameters of faba bean plant infected by root-knot nematode, *M. javanica* under greenhouse conditions during seasons 2018 and 2019

Treatments	Concentrations (ml/kg soil)	Season 2018				Season 2019			
		Fresh weight of shoots (g)	Dry weight of shoots (g)	Plant height (cm)	No. of flowers/plant	Fresh weight of shoots (g)	Dry weight of shoots (g)	Plant height (cm)	No. of flowers/plant
Cyanobacterium-based Ag-NPs	1	37.18cd	6.43de	53.5ab	20.00bcd	34.50ef	5.33cd	39.25ab	19.33bcd
	2	56.09a	7.61abcd	50.00abc	20.50bcd	52.33abc	7.20abc	41.25ab	23.33abc
	3	56.82a	8.16abc	55.00a	33.00a	56.33a	7.66ab	44.50a	27.00ab
Nostoc sp. aqueous extract (ACE)	1	44.46bc	6.43de	47.50abc	23.25bc	44.66cde	5.72bcd	39.75ab	24.33abc
	2	57.23a	8.42ab	43.50c	34.00a	55.66ab	8.16a	41.50ab	28.66a
	3	57.32a	8.52a	54.00ab	37.50a	57.55a	8.43a	44.25a	30.00a
$AgNO_3$	1	40.99bc	6.06ef	44.25c	13.25de	40.33def	5.30cd	42.00ab	13.00d
	2	38.26bcd	6.17ef	44.75c	15.50cde	43.33cdef	5.66bcd	37.75b	17.33cd
	3	45.08b	7.80abc	46.75bc	20.25bcd	45.00bcde	7.33abc	39.25ab	19.66abc
Vydate 24% SL.		43.24bc	7.23bcde	54.25ab	20.00bcd	47.66abcd	7.30abc	37.25b	19.56abc
Nematode-infected soil (control[1])		33.30d	5.14f	30.00d	10.75e	33.13f	4.66d	28.00c	13.33d
Untreated soil (control[2])		37.58cd	7.03cde	45.25c	24.50b	37.66def	7.00abc	38.25ab	24.00abc

Means values followed by a common letter(s) are not significantly different at the 5% level by DMRT

and polysaccharides in the extract of *Turbinaria conoides* and *Chlorella vulgaris* act as reducing and stabilizing agents in the biosynthesis of gold and silver chloride-NPs, respectively.

4.2 Nematicidal activity of cyanobacterium-based Ag-NPs against *M. javanica*

Our results showed that cyanobacterium-based Ag-NPs induced a significant reduction in egg hatching % and induced a marked larval mortality of *M. javanica*, in vitro. The obtained results are in accordance with previous reports which used Ag/AgCl-NP solution against *Meloidogyne incognita* [6, 18–20]. The inhibitory effect of the bioformed Ag-NPs was attributed to their physical structure (e.g., size, shape, and homogeneity) which, probably played a key role in the cell wall penetration of the nematode eggs, followed by cell dysfunction [51]. Ag-NPs induced oxidative stress and upregulation of sod-3 and daf-12 genes which caused the failure of reproduction in *Caenorhabditis elegans* worms [52]. Toxicity of Ag-NPs is not a species-specific; therefore, it can be applied to control other plant-parasitic nematodes and plant-pathogenic fungi. Its mode of action is associated with disrupting and malfunctioning of several cellular mechanisms, such as membrane permeability, ATP synthesis, and physiological response to oxidative stress in both eukaryotic [53] and prokaryotic cells [54]. Our results under greenhouse conditions over two cultivation seasons suggest the efficacy of the cyanobacterium-based Ag-NPs on the reduction of root galling and J_2 population in infected soil. A similar observation was reported by Cromwell et al. [18], Hassan et al. [19], and Nour El-Deen and El-Deeb [20]. Nevertheless, $AgNO_3$ also showed a comparable inhibitory effect against *M. javanica* under both laboratory and greenhouse conditions. In this context, Jung et al. [55] attributed the toxic effect of silver ion on *Staphylococcus aureus* and *Escherichia coli* to membrane dysfunction of the bacterial cell which possibly causes cell death.

4.3 Bio-stimulant effect of cyanobacterium-based Ag-NPs on faba bean

Based on the obtained results, cyanobacterium-based Ag-NPs seem to be a good candidate for efficient biocontrol of *M. javanica* infecting faba bean plant as well as a bio-stimulant material for plant growth. A similar finding has been reported by Iqbal et al. [25] and Nour El-Deen and El-Deeb [20]. The bio-stimulant effect by both ACE and cyanobacterium-based Ag-NPs on faba bean growth parameters is possibly due to micronutrients and many organic compounds such as auxins, gibberellins, and precursor of ethylene and betaine produced by algal cells [56]. In this context, green synthesized Ag-NPs had no plant phototoxicity when they were used to control the nematodal infection by *M. javanica* [57]. Further investigations are still needed to separate and purify of cyanobacterium-based Ag-NPs from the suspension mixture with $AgNO_3$. We assume that some unreacted Ag ions are still in solution which possibly interfere with Ag-NP action.

5 Conclusion

The present study provides a promising technique for green production of Ag-NPs using the aqueous extract of the heterocytous cyanobacterium *Nostoc* sp. PCC7524. This was likely due to its simplicity and accessibility. The study also indicated the nematicidal activity of cyanobacterium-based Ag-NPs on % egg hatching and larval mortality of the root-knot nematode *Meloidogyne javanica* in vitro and under greenhouse conditions which suggest their use in biological control of *M. javanica*. The study also highlighted the positive role of cyanobacterium-based Ag-NPs in improving some plant growth parameters cultivated in nematode-infected soil. Therefore, cyanobacterium-based Ag-NPs could be suggested as multifunctional nano-nematicide to avoid the harmful effect of chemical amendments.

Abbreviations

ACE: Aqueous cyanobacterial extract; AgCl-NPs: Silver chloride nanoparticles; Ag-NPs: Silver nanoparticles; FTIR: Fourier transform infrared spectroscopy; GI: Gall index; J_2: Second-stage juveniles; LC_{50}: 50% lethal concentration; NPs: Nanoparticles; PCR: Polymerase chain reaction; SPR: Surface plasmon resonance; TEM: Transmission electron microscopy

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Authors' contributions

SMH and ESH designed the experimental approach, analyzed the data, and wrote the manuscript. SMH contributed to the Ag-NP biosynthesis and full physicochemical characterization of Ag-NPs. ESH carried out the in vitro and in vivo studies. SMH and ESH generated all the tables and figures in the manuscript. NA helped to draft the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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