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ORIGINAL ARTICLE

Charcoal rot and root-knot nematode control on faba bean by photosynthesized colloidal silver nanoparticles using bioactive compounds from *Moringa oleifera* leaf extract

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Abstract

In Egypt, faba bean plants are severely damaged by charcoal rot, caused by *Macrophomina phaseolina* and root-knot, caused by *Meloidogyne incognita*. The current study was aimed to control these diseases using silver nanoparticles that were biologically synthesized from *Moringa oleifera* leaf extract. In this work, silver nanoparticles (AgNPs) were prepared with trisodium citrate as a reducing agent to produce chemo-AgNPs and, using an environmentally eco-friendly method, an aqueous extract of *M. oleifera* leaves under visible light radiation to produce bio-AgNPs. The obtained colloidal solutions of AgNPs were identified by UV-Visible (UV-Vis) spectral analysis and Transmission Electron Microscopy (TEM) analyses. The antifungal and anti-nematode activities of chemo- and bio-AgNPs as well as an aqueous extract of *M. oleifera* leaves were checked *in vitro* against *M. phaseolina* and *M. incognita*. The obtained results showed that bio-AgNPs were more effective than chemo-AgNPs. Under greenhouse conditions, bio-AgNPs showed a significant reduction in the incidence of damping-off and charcoal rot caused by *M. phaseolina*. This treatment also reduced the number of juveniles in the soil, the number of galls and the number of egg-masses of *M. incognita* in comparison to plants treated with nematodes. Moreover, the protein profile using SDS-PAGE was performed for determining the effect of the studied treatments on the expression of some genes compared with untreated plants the alteration in gene expression led to the formation of different proteins and the loss of others. The proteins which were formed or lost caused a significant variation in all growth and physiological parameters such as photosynthetic pigments, proline content and antioxidant enzymes of faba bean plants.

Keywords: colloidal AgNPs, *Macrophomina phaseolina*, *Meloidogyne incognita, Moringa oleifera*, *Vicia faba*

Introduction

Faba bean (*Vicia faba* L.) is the most important food legume in human nutrition worldwide (Fouad *et al.* 2013). Some countries around the world, including Egypt, produce faba bean on a large scale (Hegaba *et al.* 2014). It has been reported that faba bean plants are subject to infection by several soil-borne pathogens, inducing root rot disease, which is considered to be one of the most important limiting factors affecting

plant growth and yield (Abdel-Monaim *et al.* 2013). Among them, charcoal rot caused by *M. phaseolina* (Tassi) Goid is considered to be one of the most devastating diseases, causing serious yield loss of faba bean (Kumari *et al.* 2017). The fungus produces asexual structures, microsclerotia and pycnidia. The presence of a teleomorph is yet to be confirmed (Singleton *et al.* 1993). These sclerotia can survive in soil for 2–15 years or in root debris for longer periods (Baird *et al.* 2003). The disease is favored by hot, dry weather or when the plant is stressed by unfavorable environmental conditions (Wrather *et al.* 2001). In severe infections due to the production of fungal toxins, such as phaseolinone and vascular blockage by the fungus, organs are destroyed (Bhattacharya *et al.* 2017).

On the other hand, plant-parasitic nematodes cause severe damage to various agricultural crops. Root-knot nematodes are important vegetable pests and their host range is comprised of more than 3000 plant species. Faba bean plant is greatly affected by root-knot nematode *M. incognita* (El-Nagdi and Youssef 2004; Hamed *et al.* 2019). Silver nanoparticles can be an important alternative method to control against the most destructive root-knot nematode, *Meloidogyne* spp. (Nazir *et al.* 2019). Hamed *et al.* (2019) reported that application of AgNPs significantly reduced root galling and second-stage juveniles (J_2) of root-knot nematode *M. javanica* in faba bean.

The main way of fungal and nematode control has been the use of chemical products such as fungicides and nematicides (Abd-Elgawad and Askary 2018). Due to environmental problems as well as harmful effects on human beings caused by chemical pesticides used to control soil-borne pathogens, efforts to find alternatives to fungicides have been persistently conducted (Jeschke *et al.* 2016).

Nanotechnology has had a great impact on biological sciences such as agriculture. Among many natural compounds, silver nanoparticles (AgNPs) have attracted the attention of many researchers due to their excellent antifungal and anti-nematode activity (Min *et al.* 2009; Hamed *et al.* 2019). Silver nanoparticles can be manufactured using a variety of methods including chemical, physical, and biological approaches. The chemical method is characterized by the ease of obtaining large amounts of nanoparticles in a short period of time. However, it requires covering the nanoparticle size stabilizers (Javed *et al.* 2020). In addition, the chemicals used in the synthesis and fixation of nanoparticles are toxic and lead to non-eco-friendly by-products (Hajipour *et al.* 2012). This has led to the need to synthesize these particles using non-toxic synthetic environmental protocols and to investigate biological approaches free of the use of toxic chemicals as by-products of what is known as "green nanotechnology". To date several biological methods of synthesizing nanoparticles using microorganisms including bacteria and fungi as well as plants have been tested (Narayanan and Sakthivel 2010; Elshahawy *et al.* 2018). Since plants are free of toxic chemicals, they provide a better platform for nanoparticle synthesis and they provide natural capping agents. Moreover, the use of plant extracts also reduces the cost of isolating microorganisms and culture media, thus enhancing the cost competitive feasibility over nanoparticle synthesis by microorganisms (El-Refai *et al.* 2018; Pirtarighat *et al.* 2019). In recent years, extracts of *M. oleifera* have been found to be appropriate for the synthesis of AgNPs (Prasad and Elumalai 2011). Nanoparticles created by *M. oleifera* leaf extract have not previously been tested against plant pathogens. However, several studies reported that the use of AgNPs is limited due to their phytotoxicity to higher plants (Musante and White 2012). Therefore, it is necessary to investigate the effects of AgNPs on plant growth and development. It has been reported that application of AgNPs resulted in a significant reduction in plant elongation, fresh weight of shoot and root, and total chlorophyll content of seedlings of *Lupinus termis* L. (Dietz and Herth 2012). Also, it was found that application of AgNPs decreased carbohydrates and protein content and increased accumulation of proline in *Lupinus termis* L. seedlings (Al-Huqail *et al.* 2018). In contrast, AgNPs protected wheat plants from heat stress and improved plant growth and biomass (Iqbal *et al.* 2019). Similar inductive effects on growth parameters and chemically valuable phytochemical production were found by soaking Fenugreek (*Trigonella foenum-graecum* L.) in 1 $\mu g \cdot ml^{-1}$ of AgNPs for 5 days (Jasim *et al.* 2017). In the case of cowpea plants, root nodulation and growth promotion was significantly increased when the plants were treated with AgNPs at a concentration of 50 $mg \cdot l^{-1}$. In *Brassica* plants, the application of AgNPs showed significantly improved shoot parameters at 75 $mg \cdot l^{-1}$ (Mehta *et al.* 2016). Nanoparticles (Nps) have unique physicochemical characters and can raise the plant metabolism (Giraldo *et al.* 2014). AgNPs have a remarkable role in plant growth (Vannini *et al.* 2013), chlorophyll content (Hatami and Ghorbanpour 2013) and enzymatic activities (Mohamed *et al.* 2017). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a useful and inexpensive tool for describing the genetic structure of several plant species. The effect of AgNPs, synthesized either chemically or biologically using *M. oleifera* leaf extract on the incidence of charcoal rot and root-knot diseases of faba bean was also evaluated under greenhouse conditions. Finally, the effect of AgNPs synthesized either chemically or biologically on biochemical assays (photosynthetic pigments, proline and antioxidant enzymes) and protein profiles using SDS-PAGE techniques of faba bean were determined.

Materials and Methods

Charcoal rot pathogen and production of inocula

One virulent pathogenic isolate of *M. phaseolina* (Mp3), isolated from faba bean plants showing symptoms of charcoal rot, was used in the current study. This isolate was isolated and classified as highly virulent against faba bean based on a pathogenicity test conducted in previous studies (unpublished data). Fungal mass production of this isolate used for soil infestation under greenhouse conditions was obtained by growing the tested isolate on sorghum seed medium. This natural medium was prepared by mixing sand and sorghum seeds (1 : 1, w : w and 40% water). Then the mixture in glass bottles with cotton plugs was sterilized three times (1 h each time) at 121°C. The autoclaved medium was subsequently inoculated individually with a 5 mm disk at 20 ± 2°C for 2 weeks (Singleton *et al.* 1993).

Root-knot nematode inoculum

The culture of root-knot nematode *M. incognita* was maintained in a greenhouse on tomato plants at the Plant Pathology Department. The nematode eggs were extracted from infected roots of tomato in 0.5% sodium hypochlorite on a 25 µm sieve according to the method of Hussey and Barker (1973). The eggs were incubated at 27°C and the second stage juveniles (J_2) were hatched by using Baermann plates. Hatched J_2 were collected daily and refrigerated at 6°C for use the next day. Newly hatched juveniles from this culture were used as inoculum. Perennial patterns of adult females from tomato plant roots were used to confirm the nematode species as described by Taylor and Netscher (1974).

Laboratory experiments

Methods of preparation of silver nanoparticles (AgNPs)

Synthesis of chemo-AgNPs

A 50 ml aliquot of 1 mM $AgNO_3$ was heated and 1% of aqueous trisodium citrate (5 ml) was added dropwise to the reaction mixture with vigorous stirring. The reaction was heated until the reaction color changed to pale yellow as evidence of the formation of AgNPs. Then the mixture was left to cool at room temperature so AgNPs could act as a negative control. The colloidal solutions of silver nanoparticles were characterized by UV-visible spectral analysis (UV-Vis) and Transmission Electron Microscopy (TEM) analyses.

Synthesis of bio-AgNPs

The aqueous extract of *M. oleifera* leaves was prepared by soaking the dry leaf powder (10 g) in 100 ml distilled water. The mixture was subsequently stirred using a magnetic stirrer and heated at 100°C for 30 min. The resulting solution was filtered to obtain the aqueous extract. The chemical ingredients of the extract were identified using liquid chromatography/mass spectrometry (LC/MS). A solution of 50 ml of 1 mM aqueous $AgNO_3$ was added to the aqueous extract of *M. oleifera* leaves (50 ml). The reaction was stirred at room temperature under visible light irradiation using a light emitting diode (LED) lamp (LED bulb, Canyon, 20 Watt, λ = 400–750 nm). A change in the color of the reaction mixture was observed. The reaction was processed until the color intensity of the solution reached its maximum. The formation of a dark brown solution indicated the formation of AgNPs. After the completion of the reaction, the formed AgNPs were stored in darkness at room temperature to prevent agglomeration of the nanoparticles.

In vitro inhibitory effects of AgNPs

Against *M. phaseolina* (Mp)

The antifungal effects of chemo-, bio-AgNPs and crude extract of *M. oleifera* leaves were examined against the linear growth of *M. phaseolina* according to Min *et al.* (2009). To prepare potato dextrose agar (PDA) medium (pH 6.5–6.8), 200 g potato and 20 g dextrose were mixed in distilled water (1 l). The prepared PDA (100 ml) was poured into 250 ml conical flasks and then autoclaved. Before solidification, the above treatments were added to the growth media prior to plating in five Petri dishes (90 mm diameter) which gave the desired concentrations of 0, 0.001 and 0.05%. After 48 h of incubation, agar plugs of a uniform size (diameter 5 mm) containing cultures of *M. phaseolina* were inoculated simultaneously in the center of each Petri dish containing AgNPs. This was followed by incubation at 25 ± 2°C for 7 days. A series of Petri dishes free from AgNPs was used as control. Replicates for each treatment as well as the control treatment were performed in 10 Petri dishes. After 7 days of incubation, average colony radius and percentages of growth reduction were measured. The percentages of fungal growth reduction were determined according to the following formula:

$$\text{Fungal growth reduction \%} = \frac{C-T}{C} \times 100,$$

where: C – the mycelial growth diameter in control plates and T – the mycelial growth diameter in treated plates.

Against *M. incognita* juveniles

The efficiency of moringa crude extract (0, 10 and 50 ppm), chemo- and bio-AgNPs against the juveniles of *M. incognita* were conducted by testing different concentrations of 0, 10, 30 and 50 ppm from the stocks using sterilized distilled water as diluents. The final concentration was calculated, taking into account the volume of water containing J_2 where 1 ml from each treatment was mixed with 1 ml containing 100 ± 5 *M. incognita* J_2 to achieve the desired concentration. They were arranged in a completely randomized design at 25°C. After 24 h and 48 h of exposure, all treatments were conducted in five replicates and the average results were compared to distilled water as negative control. Also, after 48 h of exposure, the juveniles were washed with distilled water and transferred to aerated distilled water for 24 h. The juveniles which did not regain their activities and did not move when probed with a fine needle were considered "dead". On the other hand, the juveniles were considered active when they were visibly flexible and then the average percentages of nematode recoveries were determined (Cayrol *et al.* 1989). The number of active and inactive J_2 was counted with the aid of a microscope and the percentages of inactive larvae were calculated to evaluate the percentage of juvenile mortality.

Greenhouse experiments

Experiments

Four pot experiments were conducted in a greenhouse subject to natural conditions (23/12°C day/night, 60% relative humidity (RH) and the average hours of sunshine was 8 h per day) during two successive seasons at the National Research Centre (NRC), Giza, Egypt. Faba bean seeds (*Vicia faba* L.) Sakha1 were obtained from the Legume Research Institute, Ministry of Agriculture, Giza, Egypt. Plastic pots (30 cm diameter) were filled with 2 kg autoclaved sand-clay (1 : 1 v/v). Five seeds were planted in each pot in the third week of October in both seasons and seedlings were thinned to one plant per pot.

Seeds were planted in plastic pots to study the management of fungus (*M. phaseolina*) and root-knot nematode (*M. incognita*) by applications of silver nanoparticles. The concentrations of bio- and chemo-AgNPs were adjusted to 0.05% using sterilized distilled water. Seeds of susceptible faba bean cultivar, Sakha 1, were soaked in bio-, chemo-AgNPs, Topsin-M70 (fungicide), Vydate L (nematicides) and sterilized distilled water as control for 2 h.

Each experiment was divided into five main groups; the first group in all experiments was untreated seeds and the other four groups were treated with different treatments. In the first experiment, the second, third, fourth and fifth groups were soaked in Topsin, Vydate, bio- and chemo-AgNPs, respectively.

In the second experiment, the second group was planted in soil infected with fungus (*M. phaseolina*) 5 days before planting. The third, fourth and fifth groups were soaked in Topsin, and bio- and chemo-AgNPs, respectively, and then planted in soil infected with *M. phaseolina*.

In the third experiment, the second group was planted in soil and inoculated with 2000 *M. incognita* juveniles 2 weeks after germination. The third, fourth and fifth groups were soaked in Vydate, and bio- and chemo-AgNPs, respectively, then planted in soil and inoculated with *M. incognita* juveniles.

Finally, in the fourth experiment, the second group seeds were planted in soil infected by *M. phaseolina* and then inoculated with *M. incognita*. In the third, fourth and fifth groups, seeds were soaked in Topsin, and bio- and chemo-AgNPs, respectively, and then planted in soil infected by *M. phaseolina* and inoculated with *M. incognita* J_2.

Charcoal rot disease

Soils were infested individually at a ratio of 5% (w/w) with tested pathogenic fungal cultures and mixed thoroughly to ensure equal distribution of fungal inoculum. Subsequently, they were placed in formalin sterilized plastic pots (30 cm diameter) and irrigated every second day for 1 week before sowing. A set of pots was also amended with the same rate of sorghum seed medium free of fungal inoculum and reserved as control treatment. Surface sterilized faba bean seeds (using 3% sodium hypochlorite for 5 min, removed and air-dried) were planted in both infested and non-infested soil, five seeds per pot and five replicates (pots). The percentage of damping--off was recorded as the number of absent/dead seedlings relative to the number of seeds sown 30 days after planting. Percentages of dead plants due to charcoal rot disease caused by each isolate and percentages of surviving plants were assessed 90 days after planting.

Root-knot disease

Fifty days after planting faba bean plants were gently uprooted and the roots were washed and cleaned from the adhering soil particles. The contents of the pot were thoroughly mixed, and nematode juveniles were extracted from 250 g of soil by sieving and decanting (Barker 1985) and examined under a light microscope using a Hawksley counting slide. The number of galls and egg masses were counted from the whole root system. The percentage reduction in each parameter was calculated with respect to plants treated with nematodes.

Estimation of plant growth parameters

The influence of various treatments on different growth features of faba bean plants was determined. Plant length (cm), leaf, branch and flower numbers per plant, fresh and dry weights (g · plant^{-1}) of shoot systems, root length and weight (g · plant^{-1}) were taken during flowering. Each treatment was replicated five times each growing season.

Determination of photosynthetic pigments, proline and antioxidant enzyme activities

Photosynthetic pigments [Chlorophyll: (Chl a), (Chl b)] and total carotenoids (TCAR) were determined in fresh leaves which were collected from each treatment during the flowering stage (90 days from sowing) (AOAC 2016). The proline content was determined in fresh leaves which had been harvested at the flowering stage (Bates *et al*. 1973). Enzyme extraction was done according to Mukherjee and Choudhuri (1983). Both catalase activity (CAT) EC 1.11.1.6 and peroxidase activity (POX) EC 1.11.1.7 were assayed according to the method of Kar and Mishra (1976), whereas, the assay of superoxide dismutase activity (SOD) EC 1.15.1.1 was determined by measuring the inhibition of the auto-oxidation of pyrogallol according to Marklund and Marklund (1974) with slight modifications.

Estimation of total soluble proteins using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as described in Tsugama *et al*. (2011). The water-soluble proteins (W.S.P) were extracted from the leaves of *Vicia faba* from a greenhouse (17 samples from four experiments as mentioned in the greenhouse experiment) during flowering. BLUltra Prestained Protein Ladder (GeneDirex, Cat No. PM001-0500) was used as a protein marker. In this method, 10% protein separating gel was used. Protein fractionations were performed on a vertical slab gel (19.8 cm × 26.8 cm × 0.2 cm) using an electrophoresis apparatus manufactured by Cleaver, UK. The images were captured by digital camera (Sony, made in Japan) and transferred directly to the computer. The protein bands were analyzed with Total Lab program to find the molecular weight of each band in order to determine the effect of each treatment on gene expression of different genes responsible for the formation of soluble proteins in faba beans.

Statistical analysis

In this study, there were four experiments; each one had five various treatments and four replicates for each treatment. Each replicate had five pots and each pot had one plant. The experimental design followed a randomized complete block design. The average data of both seasons were statistically analyzed using one way analysis of variance (ANOVA-1) and compared by Duncan multiple range test at $p < 0.05$ using Co Stat_V6.303 (CoHort software, Monterey, CA).

Results and Discussion

Visible light-mediated synthesis of chemo- and bio-AgNPs and their characterizations

Silver nanoparticles were prepared according to a traditional method for the reduction of $AgNO_3$ by using trisodium citrate to their corresponding AgNPs to produce chemically synthesized AgNPs (chemo--AgNPs). Eco-friendly synthesis of AgNPs was achieved through the reduction of $AgNO_3$ to AgNPs during exposure of the aqueous extract of *M. oleifera* leaves to visible light. In the aqueous solution the AgNPs synthesized in this way were brown. The formation of AgNPs in the aqueous solution of *M. oleifera* leaf extract was confirmed by UV-Vis spectra and TEM analyses. In Figure 1A, the UV-Vis spectrum showed an absorption peak at 425–440 nm corresponding to AgNPs. The TEM analyses (Fig. 1B) showed that the formed AgNPs were spherical in shape and their average size was 24 nm. The chemo-AgNPs were prepared using trisodium citrate as a reducing agent. The resulting AgNPs showed UV-Vis absorption at 420 nm (Fig. 1C) and their average size was 11 nm according to the TEM image (Fig. 1D).

Representative X-ray diffraction (XRD) patterns of the bio-AgNPs were observed at 2θ angles of 38.29, 44.38, 64.56, and 77.64°, respectively, corresponding to the 111, 200, 220, and 311 planes (Shameli *et al*. 2012) (Fig. 2).

The formation of AgNPs using *M. oleifera* could be explained by the presence of some chemical constituents in the aqueous extract using liquid chromatography/mass spectrometry (LC/MS) (Fig. 3).

The presence of 2,2-dimethyl-1-pentanol, 1-hexadecanol, L-(+)-ascorbic acid 2,6-dihexadecanoate, phytol, hexadecanoic acid, oleic acid, 9-octadecenamide as major constituents along with other minor constituents (Table 1) was investigated.

These relatively different chemical constituents could be responsible for the formation of AgNPs when an aqueous solution of *M. oleifera* was added to an

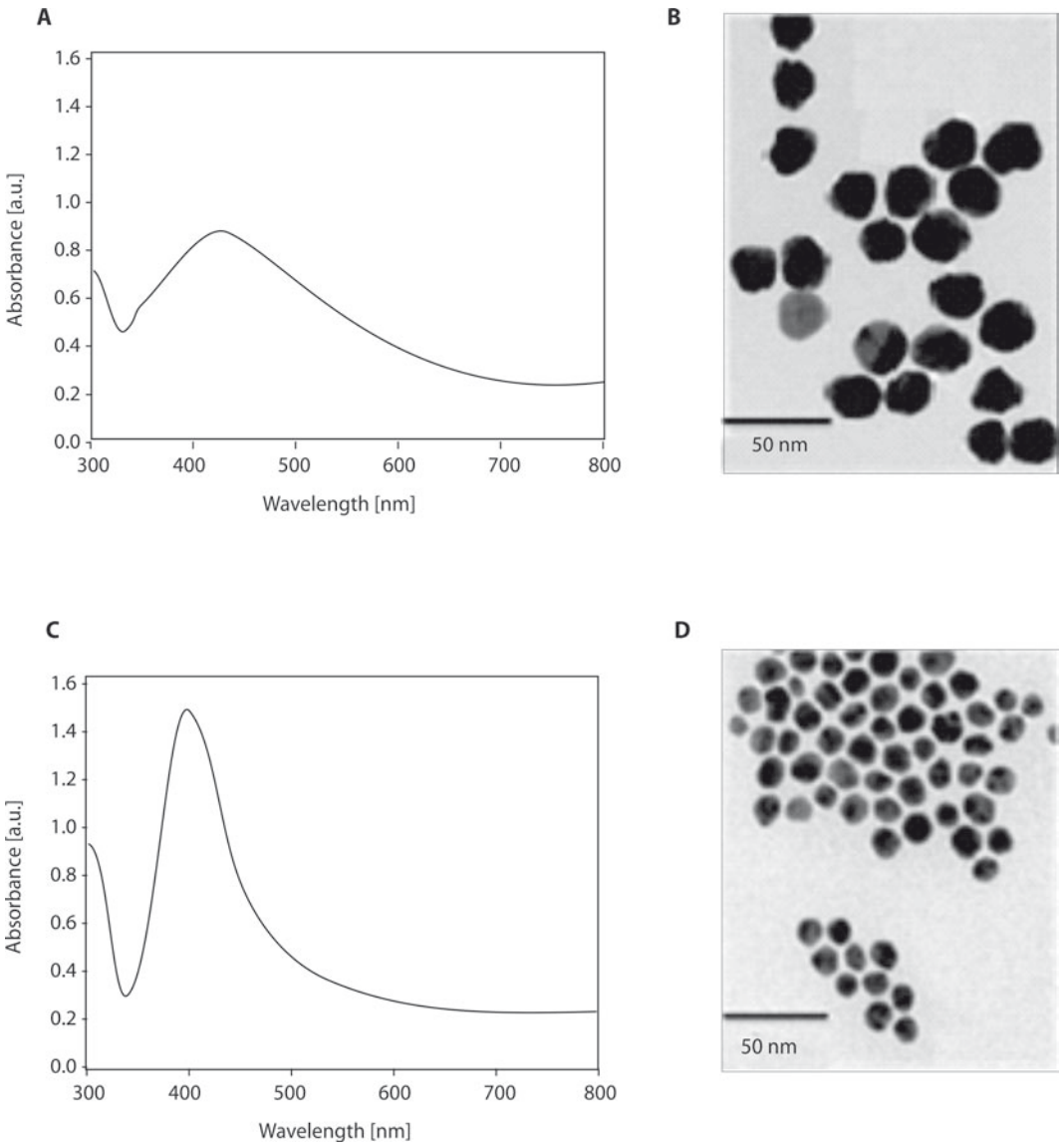


Fig. 1. (A) UV-Vis and (B) TEM image of the Ag nanoparticles formed using *Moringa oleifera* leaf aqueous extract. (C) UV-Vis and (D) TEM image of AgNPs formed using trisodium citrate

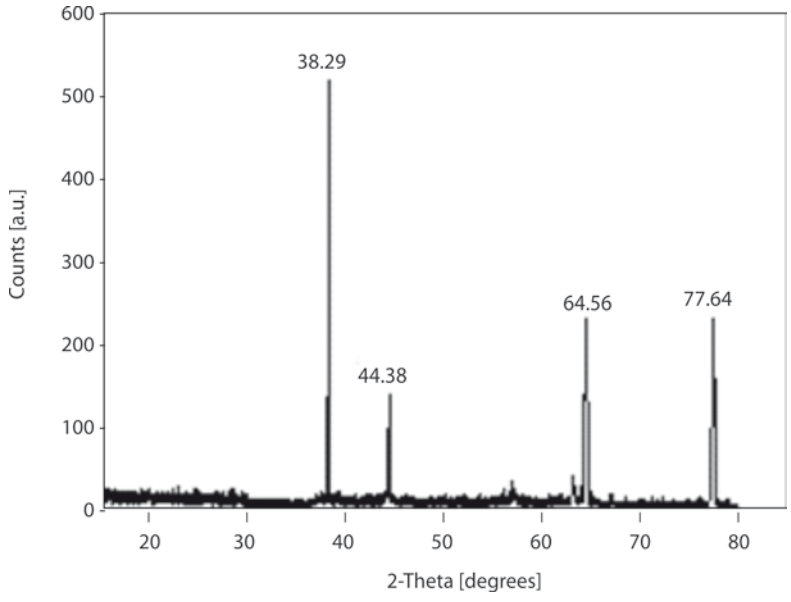


Fig. 2. X-ray diffraction (XRD) patterns of the bio-synthesized AgNPs produced

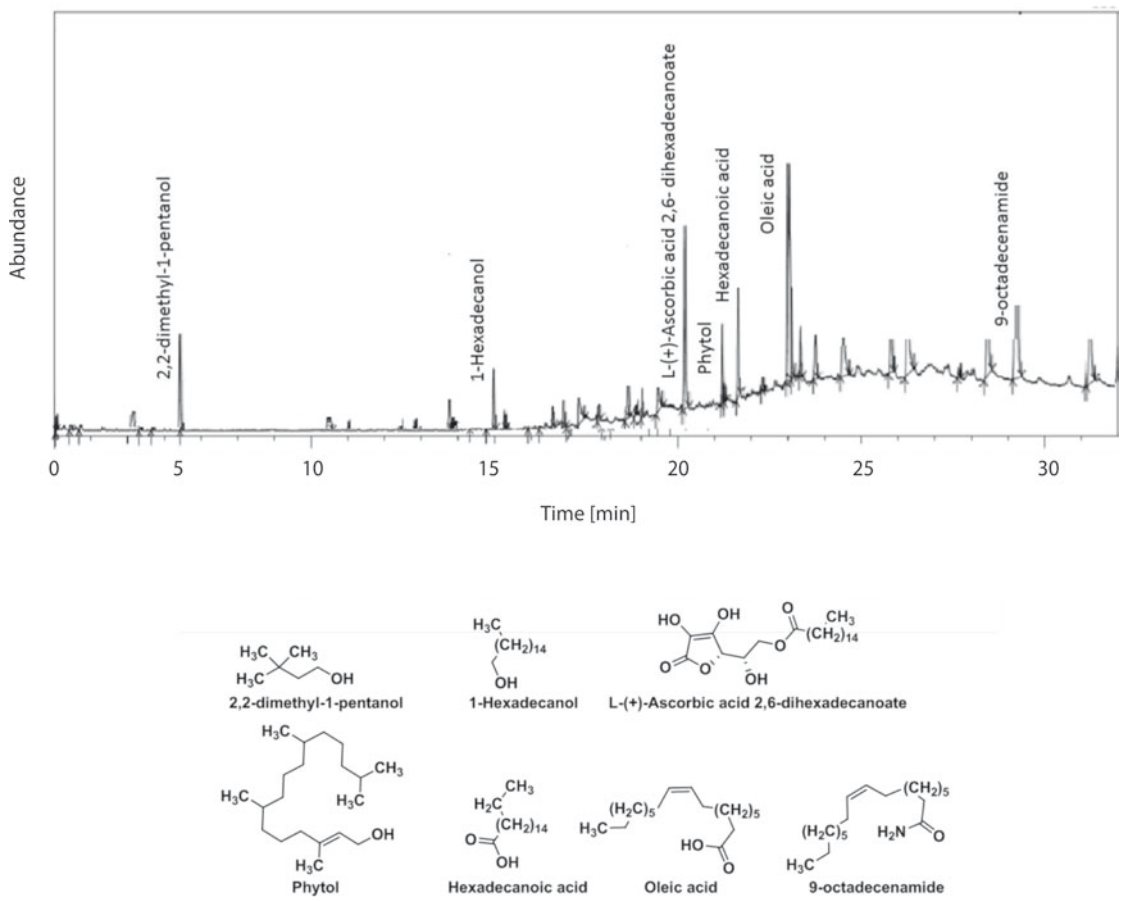


Fig. 3. Liquid chromatography/mass spectrometry (LC/MS) chromatogram of an aqueous extract of *Moringa oleifera* leaves

Table 1. Mass spectral data of an aqueous extract of *Moringa oleifera* leaves

Compound	Retention time [min]	Molecular weight [g · mol^{-1}]
2,2-Dimethyl-1-pentanol	5.011	102
1-Hexadecanol	15.173	242
L-(+)-Ascorbic acid 2,6-dihexadecanoate	20.186	414
Phytol	21.194	296
Hexadecanoic acid	21.531	270
Oleic acid	23.728	282
9-Octadecenamide	29.247	281

aliquot of 1 mM $AgNO_3$ and the produced solution was subjected to visible light irradiation. It could be postulated that the existing chemical components possessing a functional group [e.g., OH, NH_2] were responsible for the reduction of silver ions (Ag^+). Then the fatty acids [FA-COOH] present could act as capping agents that help in the stabilization of the AgNPs formed (Fig. 4).

In vitro inhibitory effect of AgNPs against *M. phaseolina* and *M. incognita* juveniles

Inhibitory effect tests of AgNPs were performed against the most virulent isolate of *M. phaseolina* on PDA plates treated with different concentrations of AgNPs (0, 10, 30 and 50 ppm) and/or crude *M. oleifera* extracts (0, 10, 30 and 50 ppm). For crude *M. oleifera*

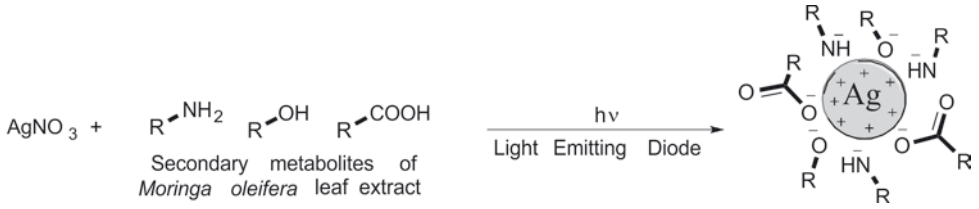


Fig. 4. Photo-synthesis of AgNPs using an aqueous *Moringa oleifera* leaf extract

Table 2. *In vitro* antifungal activity study of crude extract of *Moringa oleifera* leaves, chemo and bio-AgNPs against *Macrophomina phaseolina*

Treatment	Linear growth [mm]	Reduction [%]	% Mortality* in exposure periods	
			24 h	48 h
Moringa oleifera extract (10 ppm)	90.0 ± 0.0 a	0.0	59.20 e	59.20 e
Moringa oleifera extract (50 ppm)	90.0 ± 0.0 a	0.0	61. 67 d	70.82 d
Chemo-AgNPs (10 ppm)	90.0 ± 0.0 a	0.0	85.11 c	91.93 c
Chemo-AgNPs (30 ppm)	77.63 ± 1.45 b	17.0	68.03 e	74.19 e
Chemo-AgNPs (50 ppm)	47.5 ± 2.5 b	47.2	90.77 a	94.44 b
Bio-AgNPs (10 ppm)	90.0 ± 0.0 a	0.0	87.34 b	95.42 b
Bio-AgNPs (30 ppm)	48.32 ± 1.66 b	41.73	90.0 a	92.7 b
Bio-AgNPs (50 ppm)	00.0 ± 0.0 c	100.0	91.20 a	100.0 a
Control (0 ppm)	90.0 ± 0.0 a	–	0 f	0 f

Values are means of 10 replications. Means ± standard errors within a column followed by the same letter are not significantly different by Duncan's multiple range test at $p < 0.05$

*percentage mortality = [(mean number of living nematodes in check – mean number of living nematodes in treatment)/mean number of living nematodes in check)] × 100

extract, none of the tested concentrations exhibited an inhibitory effect and there was no statistically significant effect with the control (Table 2). Compared to the control, chemo- and bio-synthesized AgNPs showed a growth inhibition effect against *M. phaseolina*, and significant growth inhibition was observed only at 50 ppm. The lowest inhibition level (47.2%) was observed on PDA treated with 50 ppm of chemo-AgNPs (Table 2) while the highest level of inhibition (100%) was determined with 50 ppm of bio-AgNPs (Table 2).

Results presented in Table 2 indicated that all treatments exhibited a nematicidal effect against *M. incognita* at two concentrations of AgNPs and/or crude moringa extracts (10, 30 and 50 ppm) compared to distilled water as a control (0 ppm).

From the above-mentioned results, it was determined that all treatments appeared to have a nematicidal effect against *M. incognita* at different concentrations compared to distilled water as a control (0.0 ppm). Bio-AgNPs were the most effective with a mortality of *M. incognita* juveniles of 91.20% and 100%, at 24 h and 48 h, respectively, at 50 ppm concentration compared to the control (0 ppm). There was a positive relationship between nematode mortality and exposure periods for each concentration of the nanoparticles (Nazir *et al.* 2019). Generally, high concentrations achieved the highest mortality of *M. incognita* juveniles.

Greenhouse experiments

Effects of different treatments on charcoal rot

The data in Table 3 showed that all treatments significantly reduced the incidence of damping-off and charcoal rot and increased the survival of faba bean which had been artificially infested with the fungus (*M. phaseolina*) either individually or in combination with nematodes (*M. incognita*). No significant difference was observed between the same treatment under both infestation methods. Soaking faba bean seeds in bio-AgNPs reduced damping-off and charcoal rot incidence more than AgNPs synthesized chemically.

Effects of different treatments on root-knot nematodes

As seen in Table 4, there were significant nematicidal effects of chemo-AgNPs, bio-AgNPs and Vydate (nematicide), expressed by the percentage reduction of nematode parameters (number of juveniles in soil, galls and egg-masses formation compared to plants

Table 3. Effects of various treatments on damping-off, charcoal rot and survival of faba bean plants infested with *Macrophomina phaseolina* (Mp) and infested with *M. phaseolina* (Mp) and *Meloidogyne incognita* (Mi) under greenhouse conditions

Treatment	Damping-off, charcoal rot and survival plants		
	% damping-off	% charcoal rot	% plant survival
Infested plants with *M. phaseolina* (Mp)			
Untreated plants	0 d	0 d	100.0 a
F	50.0 a	31.68 a	18.32 d
F + Chemo-AgNPs	20.0 b	18.75 b	61.25 c
F + Bio-AgNPs	05.0 c	10.50 c	84.50 b
F + Topsin	05.0 c	10.50 c	84.50 b
Infested plants with *M. phaseolina* (Mp) and *M. incognita* (Mi)			
Untreated plants	00.0 d	00.0 d	100.0 a
F + N	60.0 a	32.50 a	07.50 d
F + N + Chemo-AgNPs	20.0 b	21.25 b	58.75 c
F + N + Bio-AgNPs	05.0 c	12.75 c	82.25 b
F + N + Topsin	05.0 c	11.50 c	83.50 b

Values are means of five replications. Means within a column followed by the same letter are not significantly different by Duncan's multiple range test at $p < 0.05$. Percentage of damping-off was recorded as the number of absent/dead seedlings relative to the number of seeds sown 30 days after planting. Percentages of dead plants due to charcoal rot disease and percentages of plant survival were assessed 90 days after planting. Percentage data of disease incidence (dead plants due to white rot infection) were transformed into arcsine (%) for analyses of variance, however untransformed data are presented. F = plants treated with fungus (*M. phaseolina*); F + N = plants treated with fungus (*M. phaseolina*) + nematodes (*M. incognita*)

Table 4. Effects of various treatments on root knot nematodes *Meloidogyne incognita* galls and egg-masses formation of faba bean infested with *M. incognita* and infested with both *M. incognita* and *Macrophomina phaseolina* under greenhouse conditions

Treatment	No. J_2 in soil	Reduction [%]	No. galls/root system	Reduction [%]	No. egg-masses/ root system	Reduction [%]
Plants infested with *M. incognita*						
N	124.20 a	0.00	66.40 a	0.00	46.60 a	0 .00
N + Vydate	58.40 b	53.00	36.00 b	45.78	20.60 b	55.79
N + Bio-AgNPs	60.20 b	52.98	37.00 b	43.37	21.20 b	54.50
N + Chemo-AgNPs	63.60 b	48.79	37.60 b	39.46	22.00 b	52.80
Plants infested with *M. incognita* + *M. phaseolina*						
N + F	114.80 a	0.00	53.00 a	0.00	44.40 a	0.00
N + F + Vydate + Topsin	42.80 c	62.72	20.80 d	60.75	19.40 c	56.31
N + F + Bio-AgNPs	46.20 c	59.76	28.20 c	51.70	22.60 b	49.10
N + F + Chemo-AgNPs	64.40 b	43.90	33.60 b	36.60	23.40 b	47.30

Values are average of five replicates. Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test N = plants treated with nematodes (*M. incognita*), N + F = plants treated with nematodes (*M. incognita*) + fungus (*M. phaseolina*)

treated with *M. incognita*. The obtained data showed that all treatments had the potential to significantly reduce the root–knot nematode parameters compared to plants treated with *M. incognita*.

From the results, it was observed that the effect of Vydate and bio-AgNPs application have shown similarities in the result or non-significantly ($p < 0.05$). However due to the harmful effects of nematicides, bio-AgNPs were better than Vydate from an environmental point of view. The bio-AgNPs resulted in 52.98, 43.37 and 54.50% reduction of juveniles in the soil, root galls and egg-masses, respectively.

Table 4 shows nematicidal effects of bio- and chemo-AgNPs in plants treated with *M. incognita* and *M. phaseolina* together. The application of bio-AgNPs gave 59.76, 51.70 and 49.10% reduction of juveniles in the soil, root galls and egg-masses, respectively, followed by chemoAgNPs when compared to plants treated with *M. incognita* and *M. phaseolina* together. Generally, the data showed a reduction in all nematode parameters of infected feba bean compared with plants treated with *M. incognita* + + *M. phaseolina*.

Table 5. Effects of Topsin, Vydate, bio- and chemo-AgNPs on plant growth characters and chemical contents of faba bean plants

Treatment	Shoot system						Root system	
	length [cm]	fresh wt. [g]	dry wt. [g]	branch no.	leaf no.	flower no.	length [cm]	fresh wt. [g]
Untreated plants	46.0 b	8.7 d	1.9 c	1.0 b	15.0 c	1.7 c	16.67 b	2.47 c
Topsin	44.0 b	17.2 ab	2.8 b	2.3 a	22.7 b	3.7 ab	23.00 a	7.67 a
Vydate	41.0 b	14.4 c	2.4 bc	2.0 ab	22.7 b	3.3 b	21.50 a	4.77 bc
Bio-AgNps	61.7 a	18.6 a	5.2 a	3.0 a	34.4 a	5.0 a	22.00 a	5.76 ab
Chemo-AgNPs	46.7 b	15.8 bc	2.7 b	2.0 ab	19.0 bc	4.7 ab	19.00 ab	5.75 ab

Treatment	Photosynthetic pigments [mg · g^{-1}]			Proline [µmol · g^{-1}]	Antioxidant enzymes [unit · g^{-1}]		
	Chl a	Chl b	TCAR		SOD	CAT	POX
Untreated plants	5.9 e	5.7 e	1.8 d	25.6 a	2.9 d	11.2 e	2.7 d
Topsin	8.6 b	7.8 b	2.3 b	15.0 c	3.3 c	16.8 d	2.9 c
Vydate	7.5 d	6.1 d	2.0 c	21.9 b	3.4 b	18.3 b	3.1 b
Bio-AgNPs	9.3 a	8.8 a	2.8 a	12.7 d	3.7 a	22.4 a	3.4 a
Chemo-AgNPs	8.3 c	7.5 c	2.3 b	20.3 b	3.5 b	17.4 c	2.5 e

Values are the average of three replicates. Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test
TCAR = total carotenoids; SOD = superoxide dismutase activity; CAT = catalase activity; POX = peroxidase activity; wt. = weight

Effects on growth characters and chemical contents

The first experiment: Effects of Topsin, Vydate, bio- and chemo-AgNPs on plant growth characters and chemical contents of faba bean plants

In Table 5 it can be seen that all the experimental treatments (bio-, chemo-AgNPs, Topsin and Vydate) affected various plant growth characters [shoot & root length (cm), number of leaves, branches and flowers (g · plant^{-1}), fresh and dry weights of leaves (g · plant^{-1}), fresh weight of root (g · plant^{-1})], photosynthetic pigments, proline and antioxidant enzymes. It is clear that bean plants sprayed with bio-AgNPs had a marked increase in shoot length, leaves, branch and flower numbers, fresh and dry weights of leaves, Chl a, Chl b, TCAR and the greatest amounts of SOD, CAT and POX while the Topsin treatment produced the highest values of root length and weight.

The second experiment: Effects of fungus (*M. phaseolina*) and its interactions with Topsin, bio- and chemo-AgNPs on plant growth characters and chemical contents of faba bean plants

The treatments *M. phaseolina*, *M. phaseolina* + bio-AgNPs, *M. phaseolina* + chemo-AgNPs and *M. phaseolina* + Topsin gave various differences in all growth parameters and chemical contents (Table 6). Plants treated with fungus + bio-AgNPs resulted in the greatest values of shoot length (53.3 cm), leaf (27/plant), branch (2.3/plant) and flower (4.3/plant) numbers, fresh (14.4 g) and dry (3.8 g) weights of leaves, root length (16.75 cm), root weight (2.47 g), Chl a (8.5 mg · g^{-1}), Chl b (7.8 mg · g^{-1}), TCAR (2.4 mg · g^{-1}), SOD (3.1 unit · g^{-1}), CAT (22.1 unit · g^{-1}) and POX (3.7 unit · g^{-1}). The greatest amount of proline was produced in plants treated with only fungus.

The third experiment: Effects of nematodes (*M. incognita*) and their interactions with Vydate, bio- and chemo-AgNPs on plant growth characters and chemical contents of bean plants

Plants treated with nematodes, nematodes + bio-AgNPs, nematodes + chemo-AgNPs and nematodes + Vydate produced different variations in all growth characters (Table 7). Plants subjected to nematodes + bio-AgNPs produced a significant increase in shoot length, leaves, branch and flower numbers, fresh and dry weights of leaves, root length and weight, Chl a, Chl b, TCAR, CAT, SOD and POX. The maximum value of proline (28.3 µmol · g^{-1}) was obtained from the plants exposed to nematodes only.

The fourth experiment: Effects of fungus and nematodes and their interactions with both Topsin and Vydate, bio- and chemo-AgNPs on plant growth characters and chemical contents of bean plants

The data presented in Table 8 showed the influence of various treatments (fungus + nematodes, fungus + nematodes + Topsin + Vydate, fungus + nematodes + bio-AgNPs and fungus + nematodes + chemo-AgNPs) on different growth features. Plants treated with fungus + nematodes + bio-AgNPs registered a positive trend in shoot length, leaf, branch and flower numbers, fresh and dry weights of leaves, root

Table 6. Effects of fungus and its interactions with Topsin, bio- and chemo-AgNPs on plant growth characters and chemical contents of bean plants

Treatment	Shoot system						Root system	
	length [cm]	fresh wt. [g]	dry wt. [g]	branch no.	leaf no.	flower no.	length [cm]	fresh wt. [g]
F	46.0 a	8.7 c	1.9 b	1 b	15 bc	1.7 bc	16.67 b	2.47 a
F + Topsin	34.3 b	6.9 d	1.5 b	1 b	13 c	1 c	10.25 b	1.37 c
F + bio-AgNps	37.7 b	10.2 bc	2.0 b	1.3 b	16 bc	2.3 b	10.25 b	2.16 ab
F + chemo-AgNps	53.3 a	14.4 a	3.8 a	2.3 a	27 a	4.3 a	16.75 a	2.38 ab

Treatment	Photosynthetic pigments [mg · g^{-1}]			Proline [µmol · g^{-1}]	Antioxidant enzymes [unit · g^{-1}]		
	Chl a	Chl b	TCAR		SOD	CAT	POX
Untreated plants	5.9 d	5.7 d	1.8 d	25.6 c	2.9 e	11.2 e	2.7 c
F	5.1 e	5.0 e	1.6 e	32.5 a	3.5 d	17.1 d	2.8 c
F + Topsin	6.5 c	5.9 c	2.0 c	27.2 b	4.0 b	19.6 b	3.2 b
F + bio-AgNps	8.5 a	7.8 a	2.4 a	15.5 e	4.1 a	22.1 a	3.7 a
F + chemo-AgNps	7.7 b	6.3 c	2.1 c	21.2 d	3.9 c	19.2 c	2.8 c

F = fungus (*M. phaseolina*). Values are the average of three replicates. Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test

TCAR = total carotenoids; SOD = superoxide dismutase activity; CAT = catalase activity; POX = peroxidase activity; wt. = weight

Table 7. Effects of nematodes and their interactions with Vydate, bio- and chemo-AgNPs on plant growth characters and chemical contents of bean plants

Treatment	Shoot system						Root system	
	length [cm]	fresh wt. [g]	dry wt. [g]	branch no.	leaf no.	flower no.	length [cm]	fresh wt. [g]
Untreated plants	46 b	8.7 d	1.9 b	1.0 c	15 c	1.7 c	16.67 b	2.47 c
N	36.3 c	7.2 d	1.7 b	1.0 c	10.7 d	1.3 c	17.00 b	5.37 a
N + Vydate	38 c	10.4 c	2.2 b	2.0 b	17.0 b	2.7 b	19.25 b	3.46 b
N + bio-AgNps	54.3 a	14.7 a	4.3 a	2.7 a	32.0 a	4.7 a	29.50 a	4.28 ab
N + chemo-AgNps	46.3 b	12.4 b	2.4 b	2.0 b	18.3 b	4.0 a	27.50 a	4.46 ab

Treatment	Photosynthetic pigments [mg · g^{-1}]			Proline [µmol · g^{-1}]	Antioxidant enzymes [unit · g^{-1}]		
	Chl a	Chl b	TCAR		SOD	CAT	POX
Untreated plants	5.9 d	5.7 d	1.8 d	25.6 c	2.9 d	11.2 e	2.7 e
N	4.8 e	4.0 e	1.5 e	28.3 a	3.6 c	15.6 d	3.3 c
N + Vydate	6.3 c	6.2 c	1.9 c	23.0 b	4.2 ab	18.4 b	3.5 b
N + bio-AgNps	8.6 a	7.8 a	2.5 a	17.8 e	4.5 a	22.2 a	3.9 a
N + chemo-AgNps	8.1 b	7.7 b	2.3 b	20.4 d	3.7 bc	16.4 c	3.0 d

N = nematodes (*M. incognita*). Values are the average of three replicates. Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test

TCAR = total carotenoids; SOD = superoxide dismutase activity; CAT = catalase activity; POX = peroxidase activity; wt. = weight

length and weight. In the same way, the highest values of Chl a (8.1 mg · g^{-1}), Chl b (7.8 mg · g^{-1}), TCAR (2.3 mg · g^{-1}), CAT (27.9 unit · g^{-1}), SOD (5.0 unit · · g^{-1}) and POX (4.1 unit · g^{-1}) were observed with plants subjected to fungus + nematodes + bio-AgNps treatment. On the other hand, the maximum value (39.7 µmol · g^{-1}) of proline rates was recorded under fungus + nematodes treatment.

Effects on expression of genes using SDS-PAGE protein electrophoresis

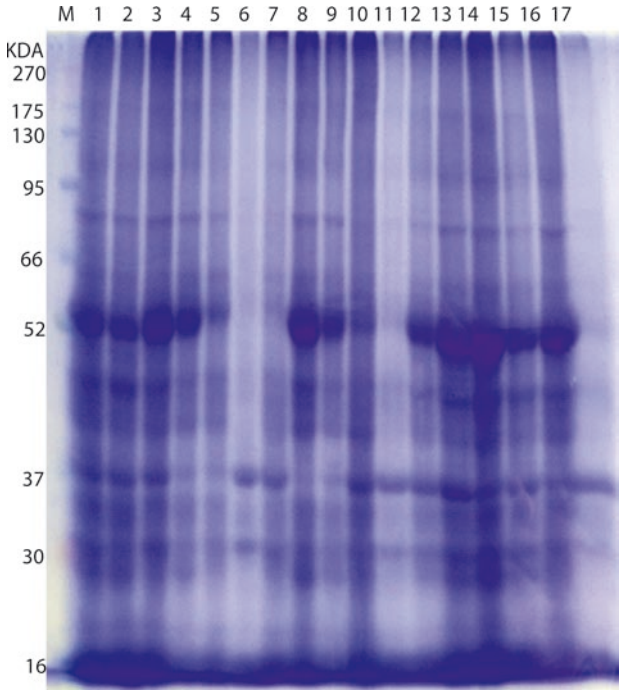
Water soluble protein profile using SDS-PAGE technique was performed for 17 samples from four experimentsin the flowering stage to determine the effect of each treatment on the expression of faba bean genes (Fig. 5).

Table 8. Effects of fungus and nematodes and their interactions with both Topsin, Vydate, bio- and chemo-AgNPs on plant growth characters and chemical contents of bean plants

Treatment	Shoot system						Root system	
	length [cm]	fresh wt. [g]	dry wt. [g]	branch no.	leaf no.	flower no.	length [cm]	fresh wt. [g]
Untreated plants	46.0 ab	8.7 c	1.9 a	1.0 b	15 b	1.7 bc	16.67 b	2.47 c
F + N	31.0 c	5.7 d	1.2 a	1.0 b	10.0 c	0.7 c	17.67 c	3.80 bc
F + N + Topsin + Vydate	44.0 b	9.4 c	1.8 a	2.0 a	21.3 b	1.8 bc	19.25 bc	4.83 b
F + N + bio-AgNPs	50.3 a	12.6 a	2.2 a	2.3 a	21.7 a	3.3 a	22.75 ab	4.86 b
F + N + chemo-AgNPs	42.7 b	11.1 b	1.9 a	2.0 a	15.7 b	2.3 ab	24.75 a	6.80 a

Treatment	Photosynthetic pigments [mg · g^{-1}]			Proline [µmol · g^{-1}]	Antioxidant enzymes [unit · g^{-1}]		
	Chl a	Chl b	TCAR		SOD	CAT	POX
Untreated plants	5.9 d	5.7 d	1.8 d	25.6	2.9 d	11.2 e	2.7 d
F + N	3.7 e	3.8 e	1.3 e	39.7 a	4.8 b	22.1 d	4.1 a
F + N + Topsin + Vydate	6.2 c	5.9 c	2.0 c	24.2	4.7 b	25.1 b	3.8 b
F + N + bio-AgNPs	8.1 a	7.8 a	2.3 a	18.4	5.0 a	27.9 a	4.1 a
F + N + chemo-AgNPs	6.4 b	6.1 b	2.2 b	21.1	4.3 c	23.4 c	3.2 c

F = fungus (*M. phaseolina*), N = nematodes (*M. incognita*). Values are the average of three replicates. Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test
TCAR = total carotenoids; SOD = superoxide dismutase activity; CAT = catalase activity; POX = peroxidase activity; wt. = weight



M: protein marker (KDa)

First experiment	Second experiment	Third experiment	Fourth experiment
1: Untreated plant	6: F	10: N	14: F + N
2: Topsin	7: F + Topsin	11: N + Vydate	15: F + N + Topsin + Vydate
3: Vydate	8: F + bio-AgNPs	12: N + bio-AgNPs	16: F + N + bio-AgNPs
4: bio-AgNPs	9: F + chemo-AgNPs	13: N + chemo-AgNPs	17: F + N +c hemo-AgNPs
5: chemo-AgNPs			

Fig. 5. Effects of studied treatments on the protein profile using SDS-PAGE on faba bean plant in the flowering stage (F = fungus; N = nematodes)

Table 9. Effects of studied treatments on protein profiles using SDS-PAGE on faba bean plant in the flowering stage: (+) present, (–) absent; F = fungus, N = nematodes

No.	MW (KDa)	First experiment					Second experiment				Third experiment				Fourth experiment			
1	280	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	230	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	185	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	+	+
5	180	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	150	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
7	110	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	103	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	94	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	90	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	80	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	78	+	+	+	–	–	–	–	+	–	+	–	–	–	+	+	+	–
14	70	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	62	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	58	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	53	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	47	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
20	45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	43	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	41	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	33	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+
27	31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	26	+	+	+	+	–	–	+	+	+	+	–	–	+	–	–	+	+
30	21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total no.		28	28	28	27	26	26	26	28	27	28	28	28	28	28	28	28	28

First experiment	Second experiment	Third experiment	Fourth experiment
1: Untreated plant	6: F	10: N	14: F + N
2: Topsin	7: F + Topsin	11: N + Vydate	15: F + N + Topsin + Vydate
3: Vydate	8: F + bio-AgNPs	12: N + bio-AgNPs	16: F + N + bio-AgNPs
4: bio-AgNPs	9: F + chemo-AgNPs	13: N + chemo-AgNPs	17: F + N + chemo-AgNPs
5: chemo-AgNPs			

There were a total of 30 bands with a molecular weight (MW) range from 21 to 280 KDa, including 23 monomorphic bands while the other seven bands were polymorphic bands (Table 9). In the first experiment, the effects of Topsin, Vydate, bio- and chemo--AgNPs were studied on the water soluble protein profile of faba bean. It was observed that the protein at MW 78 KDa was absent in bio- and chemo-AgNPs treatments while the protein at MW 26 KDa was absent only in chemo-AgNPs treatment.

In the second experiment, the effects of fungus and its interactions with Topsin, bio- and chemo--AgNPs were studied on the water soluble protein profile of faba bean. It was found that there is a protein at

MW 78 KDa present in untreated plants and bio-AgNPs treatments while protein at MW 26 KDa was absent only in infected plants with the fungus treatment. In the third experiment, the effects of nematodes and their interactions with vydate, bio- and chemo-AgNPs were studied on the water soluble protein profile of faba bean. It was found that the unique protein was present only in plants infected with nematodes at MW 185 KDa but the protein at MW 150 KDa was absent only in infected plants. Proteins at MW 90 and 33 KDa were absent in plants infected with nematodes and Vydate treatments. Also, the proteins at MW 26 KDa were absent in vydate and bio-AgNPs treatments while the protein at MW 78 KDa was present in untreated plants and plants infected with nematodes. In the fourth experiment, the effects of fungus and nematodes and their interactions with both Topsin and Vydate, bio- and chemo-AgNPs were studied on the water soluble protein profile of faba bean. It was observed that there is a protein at MW 185 KDa absent only in untreated plants. Also, there is a protein at MW 47 KDa present in both infected plants, Topsin and Vydate treatments. The protein at MW 78 KDa was absent only in chemo-AgNPs. However, the protein at MW 33 KDa was absent in Topsin, Vydat, and chemo-AgNPs treatments.

Discussion

In the current study inhibition assays indicated that bio-synthesized AgNPs was very effective against *M. phaseolina*, and *M. incognita*. It was illustrated previously that the mechanism of anti-pathogenic effect caused by AgNPs was due to the formation of free particles from the nanoparticles that lead to the destruction of the lipids present in the plasma membrane and thus the membrane loses its function and spoils (Kim *et al.* 2007). *In vitro* study proved that bio-AgNPs was highly efficient for ability the management of root-knot nematodes. The inhibitory effect of the bio-AgNPs was attributed to their physical structure (e.g., shape, size and homogeneity) which played a key role in the cell wall penetration of the nematode body (Sharon *et al.* 2010). From the results, it was demonstrated that bio-AgNPs improved the growth of faba bean under greenhouse conditions. This may be due to the role of silver nanoparticles in preventing the damage caused by charcoal rot and root-knot diseases (Sharon *et al.* 2010). It was also indicated that different effects on bean plants was recorded when each one of the treatments (bio-, chemo-AgNps, Topsin, or Vydate) was treated the plants with *M. phaseolina* and/or *M. incognita*, compared with untreated plants. Throughout plants life cycle, they are subjected to different kinds of biotic and abiotic stresses which include microbes, pathogens, salinity, water deficit, temperature, heavy metals and UV radiation. These factors limit the growth and productivity of plants in various degrees. Plants can resist these factors by different ways such as increasing proline and production of antioxidant enzymes (Hayat *et al.* 2012). In this report, the treatment of the plants with *M. phaseolina* and/or *M. incognita* with bio-AgNPs resulted in different increases of photosynthetic pigments compared with other treatments (chemo--AgNPs, Topsin, Vydate). It was noted that these results were in agreement with (Karthick and Chitrakala 2011). Important evidence that indicate on the exposure of plant to stress is the proline level (Monreal *et al.* 2007). Our results showed that the proline content was increased significantly with plants treated with *M. phaseolina* and/or *M. incognita* (Sharma *et al.* 2012). Reactive oxygen groups (ROS) are known as one of the plant stress responses, which cause a great damage by peroxidation of lipid membrane components and through direct interaction with various macromolecules (Foyer and Noctor 2005). It was shown that AgNPs affect antioxidant enzymes according to species of plants, levels and durations of AgNPs added. It was indicated that bio-AgNPs could have an efficient strategy to avoid the negative effect of stress. Furthermore, gene expression or proteins profiles of faba bean after treatment with bio-AgNPs, chemo-AgNPs, Topsin and Vydate was performed in this study. There are several studies on the mutagenic effect of nanoparticles on plants using SDS-PAGE techniques (Salama *et al.* 2019; Osman *et al.* 2020) using SDS-PAGE techniques. Various studies reported that AgNPs considered as one of the most widely used engineered nanoparticles (ENPs) due to their antimicrobial potential (Khiew *et al.* 2011; Feizi *et al.* 2013). The interaction of plant cells with the ENPs leads to the modification of plants' gene expression and associated biological pathways, which eventually affect plants' growth and development. It was observed that, there are some proteins absent in studied experiments while present in others, this may be refer to the essential needs of plant in each experiment from proteins or enzymes to keeping its survival and occurring its essential metabolic processes (Muller and Gottschalk 1973). This could be explained on the basis of mutational event at the regulatory genes that prevent or attenuate transcription. Moreover the pesticides inducted chromosomal abnormalities may lead to the loss of genetic materials (Abdelsalam *et al.* 1993). Therefore, some electrophoretic bands of some proteins can disappeared due to the loss of their corresponding genes. In the present study, the proteins profile reflected on the powerful effect of bio-AgNPs in recovered the effect of both nematodes and fungus together than the using of both commercial pesticides Topsin and Vydate together and chemo-AgNPs. Also, the Ag-NPs showed similar nematicidal effect to the reference nematicide.

Conclusions

In this study, the preparation and identification of secondary metabolites which existed in an aqueous extract of the leaves of *M. oleifera* was investigated. This extract was used in preparation of colloidal Ag-NPs (bio-AgNPs) through a photo-extracellular approach. The biological properties against charcoal rot and root knot diseases in faba bean plants compared to chemically synthesized AgNPs (chemo-AgNPs), popular fungicides and nematicides, were studied. Laboratory and greenhouse experiments were done to determine the effects of each treatment on the pathogen-parasitic nematode. The impact on growth parameters, the content of photosynthetic pigments, proline and oxidative stress of faba bean plants were demonstrated. Furthermore, the expression of genes using SDS-PAGE protein electrophoresis was reported. From these results, it was established that the as-synthesized AgNPs mediated by *M. oleifera* extract was an effective, safe fungicide and nematicide. The bio-AgNPs were demonstrated to confer significant values of protection at a concentration of ca ~50 ppm. This indicates the potential use of *M. oleifera* plants in the synthesis of AgNPs which was demonstrated to be a potent agent towards parasitic diseases in faba bean plants. In addition, bio-synthesized AgNPs were shown to be more practical and cheaper than commercial fungicides or nematicides.

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References

Abd-Elgawad M.M.M., Askary T.H. 2018. Fungal and bacterial nematicides in integrated nematode management strategies. Egyptian Journal of Biological Pest Control 28: 74. DOI: https://doi.org/10.1186/s41938-018-0080-x

Abdel-Monaim M.F. 2013. Improvement of biocontol of damping-off and root-rot/wilt of faba bean by salicylic acid and hydrogen peroxide. Mycobiology 41: 47–55. DOI: https://doi.org/10.5941/MYCO.2013.41.1.47

Abdelsalam A.Z.E., Hassan H.Z., El-Domyati M., Eweda M.A., Bahieldin A., Ibrahim S.A. 1993. Comparative mutagenic effects of some compounds using different eukaryotic systems. Egyptian Journal of Genetics and Cytology 22: 129–153.

Al-Huqail A.A., Hatata M.M., AL-Huqail A.A., Ibrahim M.M. 2018. Preparation, characterization of silver phyto nanoparticles and their impact on growth potential of *Lupinus termis* L. seedlings. Saudi Journal of Biological Sciences 25: 313–319. DOI: https://doi.org/10.1016/j.sjbs.2017.08.013

Baird R.E., Watson C.E., Scruggs M. 2003. Relative longevity of *Macrophomina phaseolina* and associated mycobiota on residual soybean roots in soil. Plant Disease 87: 563–566. DOI: https://doi.org/10.1094/PDIS.2003.87.5.563

Barker T.R. 1985. Nematode extraction and bioassays. p. 19–35. In: "An Advanced Treatise on *Meloidogyne*". Vol. II. (T.R. Barker, C.C. Carter, J.N. Sasser, eds.). North Carolina University, Graphics, Raleigh, N.C.

Bates L.S., Waldren R.P., Teare I.D. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205–207. DOI: https://doi.org/10.1007/BF00018060

Cayrol J.C., Djian C., Pijarowski L. 1989. Study of the nematocidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. Rev Nematol 12: 331–336.

Dietz K.J., Herth S. 2012. Plant nanotoxicology. Trends in Plant Science 16: 582–589. DOI: https://doi.org/10.1016/j.tplants.2011.08.003

El-Nagdi W.M.A., Youssef M.M.A. 2004. Soaking faba bean seed in some bio-agents as prophylactic treatment for controlling *Meloidogyne incognita* root-knot nematode infection. Journal of Pest Science 77: 75–78. DOI: https://doi.org/10.1007/s10340-003-0029-y

El-Refai A.A., Ghoniem G.A., El-Khateeb A.Y., Hassaan M.M. 2018. Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents. Journal of Nanostructure in Chemistry 8: 71–81. DOI: https://doi.org/10.1007/s40097-018-0255-8

Elshahawy I., Abouelnasr H.M., Lashin S.M., Darwesh O.M. 2018. First report of *Pythium aphanidermatum* infecting tomato in Egypt and its control using biogenic silver nanoparticles. Journal of Plant Protection Research 58: 137–151. DOI: https://doi.org/10.24425/122929

Fouad M., Mohammed N., Aladdin H., Ahmed A., Xuxiao Z., Shiying B., Tao Y. 2013. Faba bean. p. 113–136. In: "Genetic and Genomic Resources of Grain Legume Improvement" (M. Singh, H.D. Upadhyaya, I.S. Bisht, eds.). Elsevier. DOI: https://doi.org/10.1016/C2012-0-00217-7

Foyer C.H., Noctor G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell and Environment 28: 1056–1071. DOI: https://doi.org/10.1111/j.1365-3040.2005.01327.x

Feizi H., Amirmoradi S., Abdollahi F., Pour S.J. 2013. Comparative effects of nanosized and bulk titanium dioxide concentrations on medicinal plant *Salvia officinalis* L. Annual Research & Review in Biology 3: 814–824.

Giraldo J.P., Landry M.P., Faltermeier S.M., McNicholas T.P., Iverson N.M., Boghossian A.A., Reuel N.F., Hilmer A.J., Sen F., Brew J.A., Strano M.S. 2014. Plant nanobionics approach to augment photosynthesis and biochemical sensing. Nature Materials 13 (4): 400–408. DOI: https://doi.org/10.1038/nmat3890

Hamed S.M., Hagag E.S., Abd El-Raouf N. 2019. Green production of silver nanoparticles, evaluation of their nematicidal activity against *Meloidogyne javanica* and their impact on growth of faba bean. Beni-Suef University Journal of Basic and Applied Sciences 8: 9. DOI: https://doi.org/10.1186/s43088-019-0010-3

Hassan H.Z., Haliem A.S., Abd El-Hady E.A. 2002. Effect of pre and post treatments with ferty green foliar fertilizer on mutagenic potentiality of gokilaht insecicide. Egyptian Journal of Biotechnology 11: 282–304.

Hatami M., Ghorbanpour M. 2013. Effect of nanosilver on physiological performance of pelargonium plants exposed to dark storage. Journal of Horticultural Research 21: 15–20. DOI: https://doi.org/10.2478/johr-2013-0003

Hegaba A.S.A., Fayed M.T.B., Hamada M.M.A., Abdrabbo M.A.A. 2014. Productivity and irrigation requirements of faba-bean in North Delta of Egypt in relation to planting dates. Annals of Agricultural Sciences 59: 185–193. DOI: https://doi.org/10.1016/j.aoas.2014.11.004

Hussey R.S., Barker K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57: 1025–1028. DOI: https://eurekamag.com/research/000/002/000002412.php

Hajipour M.J., Fromm K.M., Ashkarran A.A., de Aberasturi D.J., de Larramendi I.R., Rojo T., Serpooshan V., Parak W.J., Mahmoudi M. 2012. Antibacterial properties of nanoparticles. Trends in Biotechnology 30: 499–511. DOI: https://doi.org/10.1016/j.tibtech.2012.06.004

Hayat Sh., Hayat Q., Alyemeni M.N., Wani A.S., Pichtel J., Ahmad A. 2012. Role of proline under changing environments. Plant Signaling & Behavior 7: 1456–1466. DOI: https://doi.org/10.4161/psb.21949

Iqbal M., Raja N.I., Mashwani Z.U.R., Hussain M., Ejaz M., Yasmeen F. 2019. Effect of silver nanoparticles on growth of wheat under heat stress. Iranian Journal of Science and Technology, Transactions A: Science 43: 387–395. DOI: https://doi.org/10.1007/s40995-017-0417-4.

Javed R., Zia M., Naz S., Aisid S.O., ul Ain N., Ao Q. 2020. Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. Journal of Nanobiotechnology 18: 172. DOI: https://doi.org/10.1186/s12951-020-00704-4

Jasim B., Roshmi T., Jyothis M., Radhakrishnan E.K. 2017. Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.). Saudi Pharmaceutical Journal 25 (3): 443–447. DOI: https://doi.org/10.1016/j.jsps.2016.09.012

Jeschke P. 2016. Progress of modern agricultural chemistry and future prospects. Pest Management Science 72: 433–455. DOI: https://doi.org/10.1002/ps.4190

Jurkow R., Pokluda R., Sękara A., Kalisz A. 2020. Impact of foliar application of some metal nanoparticles on antioxidant system in oakleaf lettuce seedlings. BMC Plant Biology 20: 290. DOI: https://doi.org/10.1186/s12870-020-02490-5

Karthick S., Chitrakala K. 2011. Ecotoxicological effect of *Lecanicilium* Lecanii (Ascomycota: Hypocereales) based silver nanoparticles on growth parameters of economically important plants. Journal of Biopesticides 4: 97–101.

Khiew P., Chiu W., Tan T., Radiman S., Abd-Shukor R., Chia C.H. 2011. Capping effect of palm-oil based organometallic ligand towards the production of highly monodispersed nanostructured material. p. 189–219. In: "Palm Oil Nutr Uses Impacts". Nova Science.

Kim J.S., Kuk E., Yu K.N., Kim J.H., Park S.J., Lee H.J., Kim S.H., Park Y.K., Park Y.H., Hwang C.Y., Kim Y.K., Lee Y.S., Jeong D.H., Cho M.H. 2007. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology, and Medicine 3: 95–101. DOI: https://doi.org/10.1016/j.nano.2006.12.001

Kumari M., Pandey S., Bhattacharya A., Mishra A., Nautiyal C.S. 2017. Protective role of biosynthesized silver nanoparticles against early blight disease in *Solanum lycopersicum*. Plant Physiology and Biochemistry 121: 216–225. DOI: https://doi.org/10.1016/j.plaphy.2017.11.004

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685. DOI: https://doi.org/10.1038/227680a0

Marklund S., Marklund G. 1974. Involvement of thesuperoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47: 469–474. DOI: http://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Mehta P.C.M., Srivastava R., Arora S., Sharma A.K. 2016. Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity. 3 Biotech 6: 254. DOI: https://doi.org/10.1007/s13205-016-0567-7

Min J.S., Kim K.S., Kim S.W., Jung J.H., Lamsal K., Kim S.B., Jung M., Lee Y.S. 2009. Effects of colloidal silver nanoparticles on sclerotium-forming phytopathogenic fungi. The Plant Pathology Journal 25: 376–380. DOI: https://doi.org/10.5423/PPJ.2009.25.4.376

Mohamed A.S.H., Qayyum M.F., Abdel-Hadi A.M., Rehman R.A., Ali S., Rizwan M. 2017. Interactive effect of salinity and silver nanoparticles on photosynthetic and biochemical parameters of wheat. Archives of Agronomy and Soil Science 63: 1476–3567. DOI: https://doi.org/10.1080/03650340.2017.1300256

Monreal J.A., Jimenez E.T., Remesal E., Morillo-Velarde R., Garcia-Maurino S., Echevarria C. 2007. Proline content of sugar beet storage roots: Response to water deficit and nitrogen fertilization at field conditions. Environmental and Experimental Botany 60: 257–267. DOI: https://doi.org/10.1016/j.envexpbot.2006.11.002

Mukherjee S.P., Choudhuri M.A. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Plant Physiology 58: 166–170. DOI: https://doi.org/10.1111/j.1399-3054.1983.tb04162.x

Muller H.P., Gottschelk W. 1973. Quantitative and qualitative situation of *Pisum sativum*. p. 235–253. In: "Nuclear Techniques for Seed Protein Improvement". International Atomic Energy Agency, Vienna, 430 pp.

Musante C., White J.C. 2012. Toxicity of silver and copper to *Cucurbita pepo*: differential effects of nano and bulk-size particles. Environmental Toxicology 27 (9): 510–517. DOI: http://doi.org/10.1002/tox.20667

Narayanan K.B., Sakthivel N. 2010. Biological synthesis of metal nanoparticles by microbes. Advances in Colloid and Interface Science 156: 1–13. DOI: https://doi.org/10.1016/j.cis.2010.02.001

Nazir K., Mukhtar T., Javed H. 2019. *In vitro* effectiveness of silver nanoparticles against root-knot nematode (*Meloidogyne incognita*). Pakistan Journal of Zoology 51: 2077–2083. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.6.2077.2083

Osman S.A., Salama D.M., Abd El-Aziz M.E., Shaaban E.A., Abd Elwahed M.S. 2020. The influence of MoO_3-NPs on agro-morphological criteria, genomic stability of DNA, biochemical assay, and production of common dry bean (*Phaseolus vulgaris* L.). Plant Physiology and Biochemistry 151: 77–87. DOI: https://doi.org/10.1016/j.plaphy.2020.03.009.

Pirtarighat S., Ghannadnia M., Baghshahi S. 2019. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment. Journal of Nanostructure in Chemistry 9: 1–9. DOI: https://doi.org/10.1007/s40097-018-0291-4

Prasad T., Elumalai E. 2011. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine 1: 439–442. DOI: https://doi.org/10.1016/S2221-1691(11)60096-8

Salama H.M.H. 2012. Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). International Research Journal of Biotechnology 3: 190–197. DOI: http://www.interesjournals.org/IRJOB

Sharma P., Bhatt D., Zaidi M.G.H., Saradhi P.P., Khanna P.K., Arora S. 2012. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Applied Biochemistry and Biotechnology 167: 2225–2233. DOI: https://doi.org/10.1007/s12010-012-9759-8

Sharon M., Choudhary A.K., Kumar R. 2010. Nanotechnology in agricultural diseases and food safety. The Journal of Phytology 2: 83–92.

Singleton L.L., Mihail J.D., Rush C.M. 1993. Methods for research on soilborne phytopathogenic fungi 85 (1): 140–141. DOI: http://doi.org/10.2307/3760494

Vannini C., Domingo G., Onelli E., Prinsi B., Marsoni M., Espen L., Bracale M. 2013. Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. PLoS One 8: e6875. DOI: https://doi.org/10.1371/journal.pone.0068752

Wrather J.A., Anderson T.R., Arsyad D.M., Tan Y., Ploper L.D., Puglia A.P. 2011. Soyabean disease loss estimates for the top 10 soybean producing countries. Canadian Journal of Plant Pathology 23: 115–121. DOI: https://doi.org/10.1094/PDIS.1997.81.1.107