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(Review Article)

The role of silver nanoparticles biosynthesized from *Citrus aurantium* Leaves in some Vegetative and physiological characteristics of fenugreek plant *(Trigonella foenum-graecum* L.*)*

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Abstract

The research was crried out in the plant tissue culture laboratory and the green house of Department of plant Biotechnology in College of Biotechnology/AL-Nahrain University. The aim of it, is the Green-synthesis of silver nanoparticles by using an aqueous extract of *Citrus aurantium* leaves and characterizied the silver nanoparticles, using visible method depend on colour changing of the solution(mixture of aqueous extract of *C. aurantium* leaves and 1mMol of $AgNO_3$ under 60C°), UV-Visible spectroscopy method and Atomic Force Microscopy (AFM), then study the effects of foliar spray of of AgNPs on some vegetative and physiological characteristics of Fenugreek Plant (*Trigonella foenum-graecum* L.), such as fresh and dry weight, length of shoot, carbohydrates, proteins and proline. The result showed the positive effect of using AgNPs on these characteristics.

Keywords: *Citrus aurantium*; Bio-synthetized silver nanoparticles; *Trigonella foenum-graecum*; Green-synthesis

1. Introduction

Citrus aurantium is the Latin name for a plant commonly referred to as bitter orange, sour orange. It is a source of synephrine and several other biogenic amines, as well as other bioactive phytochemicals. [1]

Bitter orange *C. aurantium* is a member of the Rutaceae family, a hybrid between Pummelo *Citrus grandis* and Mandarin *Citrus reticulata*.[2]

Fenugreek *Trigonella foenum-graecum* is a perennial herb with a height between 20-60 cm. It has a hollow stem with small branches branching out from it, each of which bears three long serrated leaves at the end. From the base of the leaf stem appear small yellow flowers that turn into fruits in the form of hooked pods. Each pod is about 10 cm long and contains seeds that are somewhat similar in shape to the kidney. Greenish yellow colour. There are two types of circuit, the regular municipal circuit with a yellowish color, and the red circuit, known as the horse circuit, and they differ greatly. The ring in question here is the normal yellow ring. The part used medicinally from the fenugreek plant is the seeds and germinated seeds. In Arab medicine, the fenugreek plant was considered of great importance, so that some of them went to the aforementioned saying, “If people knew its benefits, they would buy it by its weight in gold.”[4]

In fact, the seeds of fenugreek have included many healing properties, and this is evidenced by the ancient medical uses of the Arabs. They were described as hot and dry, and were used as a result of their saliva to soften solid tumors. Internally, they were used cooked with dates and figs to treat chest pain, cough, asthma, shortness of breath, expulsion of gases, and diuresis.[3]

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It was also used to facilitate childbirth, purify the uterus, and treat hemorrhoids. It was also considered a fattening and tonic food for the body. It was also given to treat spleen tumors, while using a mixture of its flour and vinegar topically at the same time. As well as for the treatment of constipation and joint pain and others.[3]

In modern medicine, it was found from the analysis of fenugreek (Al-Rajaa) that it is rich in protein, phosphorus, and starchy substances, and it is similar in that to cod liver oil. It also contains choline and triniline, which are similar in composition to nicotinic acid, which is one of the B vitamins. It also contains inositol and Iron and its seeds contain resin, fixed oils, and volatile oil similar to aniseed oil [6]

Medicinal plants play an important role in human life, as they come second after basic food plants in terms of human needs because of their therapeutic and nutritional properties [6]

Nanotechnology has proven its importance in the agricultural field and in the various stages of production, as it contributed to improving the ability of plants to absorb Nutrients I have found many solutions to farming problems with catalytic improvements compared to traditional farming systems. Nano is defined as a very small unit of measurement representing one billionth of a meter, that is, it is impossible to see it with the naked eye or simple magnifiers.

Nano is used in atomic measurements to determine the sizes of particles of matter in it [4]

Nanoparticles possess distinct chemical, physical, electronic and optical properties that can change based on the size and shape of the particles, allowing their use in various applications and fields.

Plant extracts are complex in composition, containing various chemical compounds arising in the process of plant life, and transferring into solution during extraction. In the process of the synthesis of Ag-NP according to the general scheme Ag(I)→Ag, the chemical compounds contained in the extract can fulfill three functions:

- Act as a reducing agent of silver(I) compounds to elemental silver,
- Act as an agent that has a specific influence on the formation of a certain shape and size of Ag-NP due to inhibition of the growth of certain faces of the nanocrystals of these nanoparticles and
- Act as a stabilizer formed during the synthesis of Ag-NP.

Researchers have explored the use of silver nanoparticles as carriers for delivering various payloads such as small drug molecules or large biomolecules to specific targets. [2]

Once the Ag-NP has had sufficient time to reach its target, release of the payload could potentially be triggered by an internal or external stimulus. [2]

Aim of study:

The research is aimed to known the role of biosynthetized silver nanoparticles in different concentrations, which synthetized by using aqueous extract of *Citrus aurantium* leaves on some vegetative and physiological characteristics of Fenugreek plant (*Trigonella foenum- graecum* L.).

1.1 Nanoparticles

In recent years Nanoparticles are involved with new applications in areas like information and communication technology, power engineering, industrial engineering, environmental engineering etc. For decades some Nano scale materials have been involved whereas others are newly discovered are used as sunscreens and cosmetics, textiles, coatings, sports goods, explosives, propellants and pyrotechnics or their applications are currently under development. All in all, the number of nano products and methods of their use increase continually[4].

projects are available some of the Nanoparticles get synthesized are cost effectiveness. For example Nanoparticles synthesis using plant sources is largely adopted due to its eco-friendly nature and cost effectiveness etc.[4]

There are several purposes for the development of nanofood. These include improvement of food safety, enhancement of nutrition and flavor, and cutting production and consumer costs. In addition, nanofood provides various benefits by which include health promoting additives, longer shelf lives and new flavor varieties The application of nanotechnology in food is rapidly emerging and is involving all areas of the food chain from agricultural applications to food processing and enhancing bioavailability of nutrients.[5]

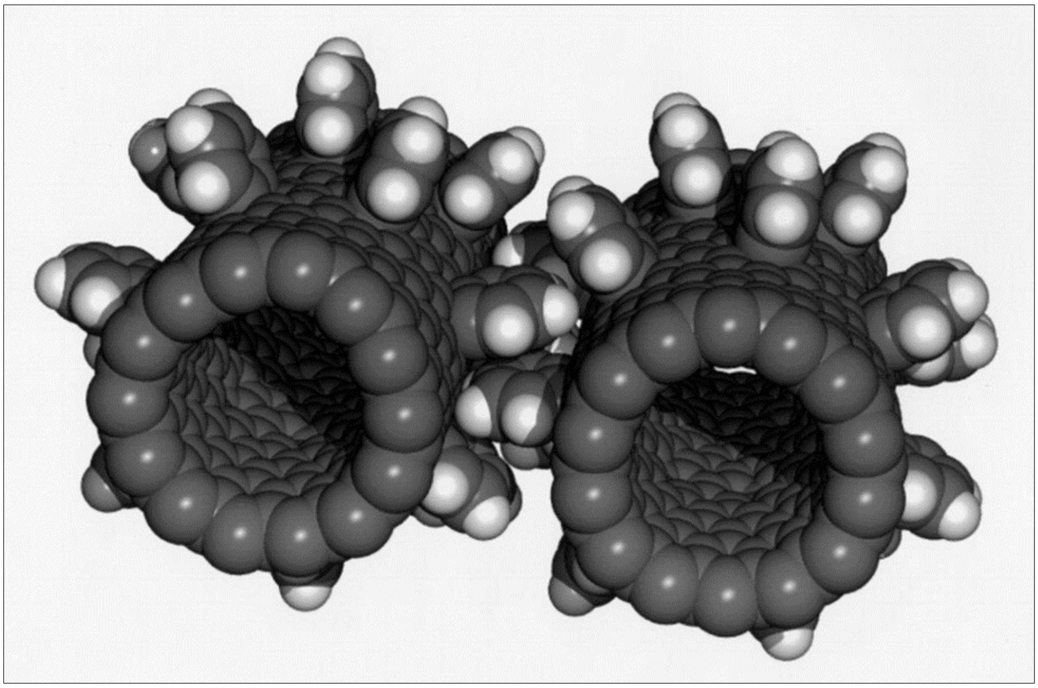


Figure 1 Nanopartical shape

1.2 Green Synthesis Methods

Green Synthesis Using Plants and Plant Extract as a Medium. One of the first approaches of using plants as a source for the synthesis of metallic nanoparticles was with alfalfa sprouts [26], which was the first report on the formation of AgNPs using a living plant system. Alfalfa roots have the capability of absorbing Ag from agar medium and transferring them into the shoots of the plant in the same oxidation state. In the shoots, these Ag atoms arranged themselves to form nanoparticles by joining themselves and forming larger arrangements. In comparison to bacteria and fungi, green synthesis using plants appears to be faster and the first investigations demonstrate that synthesis procedures are able to produce quite rapidly AgNPs. Shankar et al. showed that using *Geranium* leaf takes around nine hours reaching 90% reaction compared to the 24 to 124 hours necessary for other reactions reported earlier [27]. Therefore, the use of plant extracts in green synthesis has spurred numerous investigations and studies up till now. It was demonstrated that the production of metal nanoparticles using plant extracts could be completed in the metal salt solution within minutes at room temperature, depending on the nature of the plant extract. After the choice of the plant extract, the main affecting parameters are the concentration of the extract, the metal salt, the temperature, the pH, and the contact time [28].

In addition to the synthesis parameters, the main issue is the choice of the plant from which the extract could be used. The advantages of using plants for the synthesis of nanoparticles are that the plants are easily available and safe to handle and possess a large variety of active agents that can promote the reduction of silver ions. Most of the plant parts like leaves, roots, latex, bark, stem, and seeds are being used for nanoparticle synthesis [10]. The most important point is the active agent contained in these parts which makes the reduction and stabilization possible. Ecofriendly plant extracts contain biomolecules, which act as both reducing and capping agents that form stable and shape-controlled nanoparticles. Main compounds which affect the reduction and the capping of the nanoparticles are biomolecules such as phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds. However, quinol and chlorophyll pigments, linalool, methyl chavicol, eugenol, caffeine, theophylline, ascorbic acid[12]

1.3 Fenugreek-Plant description

Fenugreek (*Trigonella foenum-graecum* L) is an herbaceous annual plant grown for its leaves and seeds. It is one among the oldest medicinal plants in the world. The fenugreek plant may have a single stem or may be branched at the stem base. The plant has an erect growth habit and a strong, sweet aroma. The leaves of the plant are small and trifoliate with oval leaflets, which are green to purple in color. The plant produces solitary pale white or purplish flowers and a straight or occasionally curved yellow pod which houses the seeds. Between 10 and 20 seeds are produced per pod and they are small, smooth and brown, each divided into two lobes. Fenugreek can reach a height of 60 cm (23.6 in) and as an annual, survives only one growing season.

Figure 2 Fenugreek-Plant

1.4 *Citrus aurantium* Leaves

Figure 3 *Citrus aurantium* Leaves

Citrus fruits have been a commercially important crop for thousands of years. In addition, Citrus essential oils are valuable in the perfume, food, and beverage industries, and have also enjoyed use as aromatherapy and medicinal agents. The genus *Citrus* is one of the ancient, most traded, and most popular crops. The earliest records of its cultivation date back to 2100 BC.[25]

The origin of *Citrus* is still controversial; however, it is believed to have originated from Southeast Asia. *Citrus* is grown widely all over the world for its numerous health benefits. Citrus fruits are consumed as a fresh fruit desert or used for making juice and jam. They are an excellent source of vitamins, especially vitamin C. Processing Citrus fruits results in a significant amount of waste (peels, seeds, and pulps), which accounts for 50% of the fruit Citrus waste is a valuable source of d-limonene, flavonoids, carotenoids, dietary fibers, soluble sugars, cellulose, hemicellulose, pectin, polyphenols, ascorbic acid, methane, and essential oils.[22]

Citrus leaves are also a significant source of bioactive constituents including flavonoids, ascorbic acid, and phenolic constituents that are recognized as natural antioxidants [24]. *C. aurantium* leaves can be used in pharmaceutical industries since they can be integrated in drug formulations [25].

Many studies were done on *C. aurantium* leaves; they are known to *contain* various essential oils including mainly limonene, linalool, α-terpineol, and linalyl acetate [26,27]. Phytochemical analysis of *C. aurantium* leaves reflected the presence of several compounds including flavanoids, phytosterols, carbohydrates, saponins, volatile oil, tannins, ter-penoids, and proteins [28]. In the same study, 35 compounds were identified after reading the GC-MS profile of *C. aurantium* essential oil obtained through hydrodistillation. The major essential oils identified were eucalyptol (43%), sabinene (17%), β-linalool (15%), α-terpineol (8%), α-pinene (1.3%), β-myrcene (1.2%), 4-terpineol (1.1%), β-pinene (1%), D-limonene (1%), and O-cymene (1%) [28].

2. Biosynthesis of silver nanoparticles from aqueous extract of *Citrus aurantium* leaves

2.1 Preparation of *C. aurantium* leaf extract

Leaves were cleaned well with running water, soaked for 30 min in D.W., dried well using dry air, cut into small pieces using sterilized scissors.15gm of small pieces of leaves+150 ml of D.W. at 60C̊ with shaking for 15 min. Allowing to cool at room temperature Filtered solution with filtered paper. The extract stored at 4C̊ to be utilized in the following step:

Figure 4 Leaves were cleaned and soaked

2.2 Greensynthesis of silver Nanoparticles(AgNPs)

2.2.1 Preparation of 1mM of Ag NO3 solution

To prepare100ml of 1mM of $AgNO_3$ by use Molarity law

$$M = W/M.wt \times 1000/V$$

W: weight
M.wt : Molecular weight
V : volume in Liter

$0.001 = W/169.87 \times 1000/100 = 0.017gm$

Dissolve (0.017gm of $AgNO_3$) in 100m of D.W.

2.2.2 Synthesis of Silver Nanoparticles:

In (flask 250ml), 10 mL of Aqueous extract of Citrus aurantium leaves was mixed with 90 ml of a concentration of 1mM freshly prepared silver nitrate aqueous solution ($AgNO_3$) and constantly stirred using a hot plate magnetic stirrer, with the rotation speed of 200 rpm at 60Č under dark conditions. After 30 min, the mixture solution turned turbid and became Reddish-brown, and the color of the colloidal suspension changed from yellow to brown, suggesting the formation of silver nanoparticles. In order to purify the AgNPs by removing the extract, the suspension was centrifuged three times at 15,000 rpm for 20 min to obtain a dark brown Precipitate and washed twice with double sterilized water, Finally, the powder precipitate was dried to yield silver nanoparticles

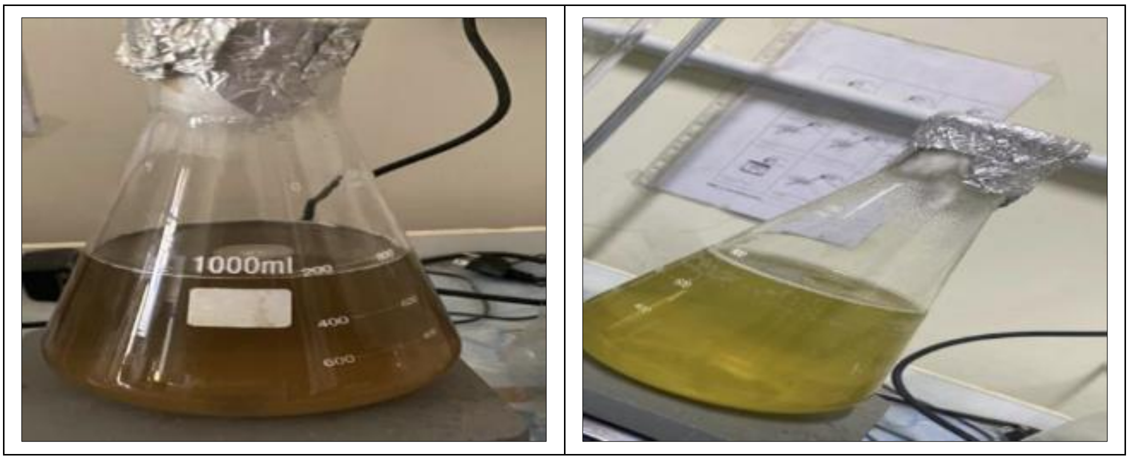


Figure 5 Aqueous solution (**$AgNO_3$**) extract of *Citrus aurantium* leaves

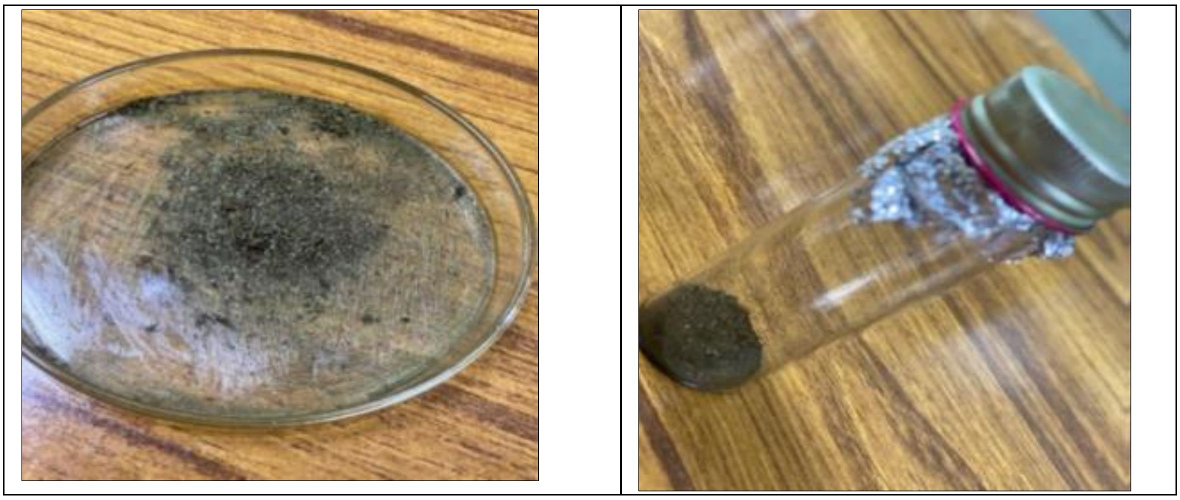


Figure 6 The powder to yield silver nanoparticles

3. Characterization of nanoparticles

By using: atomic force microscopy (AFM) and UV-visible spectr spectroscopy (UV-vis).

3.1 Preparation of concentration of AgNPs

Preparaied concentration: 100mg/ml, then Prepaired the other concentrations from this concentration: (0.5 , 1, 2 mg/ml).

3.2 Seed germination experiment:

- I took 2 petri dishes, added 25seeds of fenugreek seeds , and added filter papers under sterile conditions using a growth chamber , germination is done.
- Seed germination percentage(GP%) = GN× 100 / SN
- GN : total number of germinated seeds , SN : total number of tested seeds.
- Seed germination percentage(GP%) = 20× 100 / 25
  (GP%) = 80

Figure 7 Seeds germination experiment

4. The Field experiment

4.1 Experimental design

The experiment was designed according to the Randomized Blocks Completely Design (RBCD) in one factor, which is the plant with four levels representing to different concentrations of AgNPs, and thus the number of experimental units becomes 8.

4.2 Field experiment

Figure 8 Spraying Seeds of Fenugreek plant

The experiment was carried out in 14/12/2022 in the glass house of the Plant technology department of College of Biotechnology in 2022-2023 growing season. Seeds of fenugreek plant were cultured in pots contaning soil in glass house, with two replicates for each concentration Ag NPs treatments (0.0, 0.5, 1.0, and 2.0mg/ml). The concentration (0.0 mg/ml) uses as control for Comparison. Seeds were brought from certified selling centers to ensure the purity and free of pathogens and contaminants. Germinated seeds and seedlings were monitored, cared for and irrigated. The first spraying process after 26 days of cultivation(4-26)leaves

The second one after one week. We take samples for analysis vegetative growth after 75 days.

5. The phenotypic and physiological characteristics

5.1 Shoot length(cm)

Three plants were measured for each experimental unit, as they were measured from the level of the soil surface to the top of the plant using a graduated ruler, then the average for those lengths.

5.2 Fresh Weight gm of shoots /plant

The average of fresh weight of shoots of the plant was calculated using sensitive balance for three plants per experimental unit.

5.3 Dry weight gm/plant

The average of dry weight of three plants shoots for each experimental unit was calculated using the sensitive balance, after the dring of vegetative samples in an electric oven at a temperature of 65 °C for 48 hours until the dry weight was stable.

5.4 Estimation the percentage of total protein (%) in vegetative parts(shoot)

The percentage of total protein was estimated for the shoots, as the weight of 0.5 gm was taken of the shoots, then digested

and determined the nitrogen concentration using a micro- kjeldahl-, and after the protein percentage was estimated according to the Vopyan (1984) method.

According to the following equation:

The percentage of protein=N% × 6.25

6.25 : represents to the percentage of nitrogen in amino acid.

The analyzes were carried out in the laboratories of the Science college/Baghdad university.

5.5 Estimation of carbohydrates in vegetative parts(shoot)

The standard curve for carbohydrates was prepared, then carbohydrates were estimated by Herbert *et al*., (1971), which is called (phenol- sulfuric acid method). Color intensity was measured by a spectrophotometer at wavelength 488 nm.

The analyzes were carried out in the laboratories of the Science college/Baghdad university.

6. Results and Discussions

6.1 Characterization of Nanoparticles

In this study, the prepared silver nanoparticles was characterized by using UV-VIS spectroscopy", Atomic Force Microscope (AFM), Fourier transform infrared spectroscopy (FTIR). "AgNPs were synthesized using green method, which *Citrus aurantium* leaves extract was used as a reducing and stabilizer agent to reduce silver ion Ag+ in $AgNO_3$ to silver nanoparticles (Ag^{o}), as capping and stabilizer agent. After the addition of *C. aurantium* leaves extract to $AgNO_3$ and heated to 60̊ C. The mixture color was changed from colorless to pale yellow, which indicated initial reduction, then to redish brown with the time at 60̊C after 60minutes, which due to excitation of surface Plasmon resonance (19).

6.2 Visual Observation and UV-Vis Spectral Study

UV-Visible spectroscopy was used to examine size and shape of nanoparticles in aqueous suspensions (21). Formation and stability of prepared AgNPs in sterile distilled water was approved by UV-Vis spectrophotometer in a range of 200-800 nm of wavelength". Once *C. aurantium* leaves extract was mixed with $AgNO_3$, the reduction reaction of Ag^+ ions to Ag^o was observed by measuring UV-Vis spectrum for the reaction media. (Figure 10) showed the recorded UV-Vis spectra after the completion of reaction. the UV–visible spectrum of AgNPs showed an absorption peak at 278 nm, corresponding to the surface Plasmon resonance band of silver nanoparticles.

A	B	C

A- leaf extract of *C. aurantium* only , 1mMol of Ag NO3; B- Mixture of leaf extract of *C. aurantium* and 1mMol of Ag NO3, after 5 minutes ; C- Change the mixture after the addition of *C. aurantium* leaves extract to AgNO3 and heated to 60˚ C.

Figure 9 Change of leaf extract of *C. aurantium* after adding $AgNO_3$

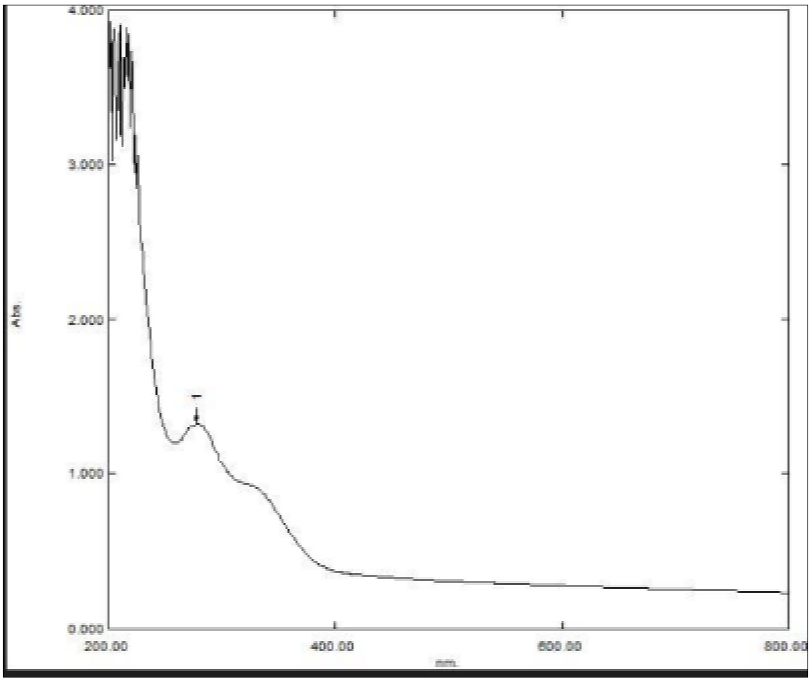
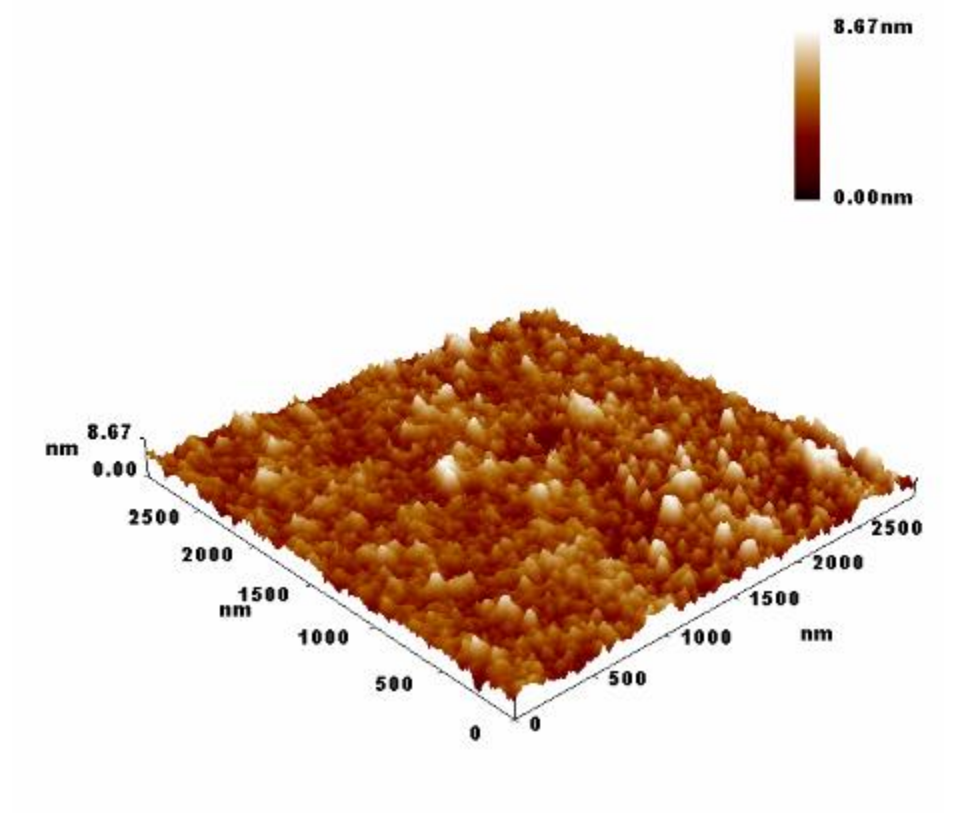


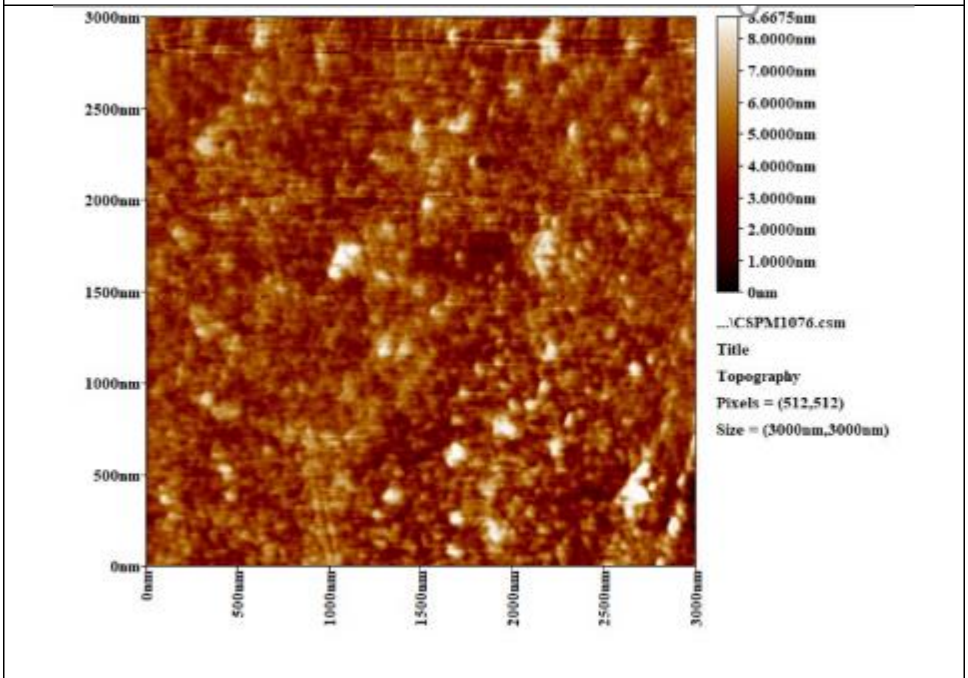
Figure 10 UV-vis spectra showing absorption of AgNPs

6.3 Atomic Force Microscopy (AFM)

AFM was one of the primarily tools for measuring, imaging and manipulating matter at the nanoscale(10); it was employed to characterize the size and morphology of AgNPs. (Figure 11) showed AFM images and corresponding size distribution of prepared AgNPs, and it was found that the size of nanoparticles is 8.67nm, roughness average : 0.83nm, and the average diameter about 42.08nm.



A



B

Figure11 (A and B) AFM images of silver nanoparticles

7. The effects of AgNPs on phenotypic and physiological characteristics

7.1 The effects of AgNPs on shoot length(cm)

Spraying with silver nanoparticles led to an increase in average of shoot length of plant at each concentration compared with control, and the highest one at the concentration1m/gml, as it appears in Table (1).

7.2 The effects of AgNPs on Fresh Weight gm of shoots (gm /plant)

The results of table No. (1) showed a decrease in the average of fresh weight of the vegetative part of the fenugreek plant compared with the control, and it was the lowest value when treated at concentration1mg/ml.

7.3 The effects of AgNPs on Dry weight gm/plant

The results of the table (1) indicated that there was a decrease in the average of dry weight of the vegetative part compared with the control, and it was the lowest value when treated with concentration2mg/ml.

Table 1 The effects of AgNPs on shoot length(cm), Fresh and Dry weight of shoots gm/plant.

Concentration (mg/ml)	shoot length cm/plant	Fresh Weight of shoot gm/plant	Dry weight of shoot gm/plant
C1: control: 0.0	28	2.54	0.68
C2: 0.5	33.5	2.36	0.59
C3: 1	38.5	1.36	0.68
C4 : 2	32	1.37	0.38

7.4 The effects of AgNPs on the percentage of total protein (%) in vegetative parts(shoot)

The results of the table (2) confirmed the presence of an increase in the average percentage of protein when treated with the three different concentrations compared with control, as there was an increase when treated at concentration(1mg/ml) followed by concentration(0.5mg/ml) and then at concentration(2mg/ml) compared with control.

7.5 The effects of AgNPs on the carbohydrates in vegetative parts(shoot)

The results of the table (2) showed an increase in the average percentage of carbohydrates at concentration 0.5mg/ml, while a decrease occurred at concentrations 1mg/ml and 2mg/ml compared to control.

Table 2 The effects of AgNPs on the percentage of total protein (%) and carbohydrates in vegetative parts(shoot)

Concentration (mg/ml)	Protein %	Carbohydrates%
C1: control: 0.0	27.05	15
C2: 0.5	28.65	15.05
C3: 1	29.2	13.4
C4 : 2	27.6	13.55

8. Conclusion

Spraying with AgNPs led to an increase shoot length of plant and decrease the fresh and dry weight of the vegetative part of it compared with control, whereas an increase in the percentage of protein when treated with the three different concentrations. An increase in percentage of carbohydrates at concentration 0.5mg/ml, while a decrease at concentrations 1mg/ml and 2mg/ml compared to control.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no conflict of interest.

References

[1] García-Salas, P.; Gómez-Caravaca, A.M.; Arráez-Román, D.; Segura-Carretero, A.; Guerra-Hernández, E.; García-Villanova, B.; Fernández-Gutiérrez, A. Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of whole-lemon powder. Food Chem. 2013, 141, 869–878. [CrossRef]

[2] Putnik, P.; Bursa´c Kovaˇcevi´c, D.; Režek Jambrak, A.; Barba, F.J.; Cravotto, G.; Binello, A.; Lorenzo, J.M.; Shpigelman, A. Innovative "green" and novel strategies for the extraction of bioactive added value compounds from citrus wastes—A review. Molecules 2017, 22, 680. [CrossRef]

[3] USDA-Foreign Agricultural Service (USDA-FAS). Citrus: World Markets and Trade; Foreign Agricultural Service: Washington, DC, USA, 2020.

[4] Lv, X.; Zhao, S.; Ning, Z.; Zeng, H.; Shu, Y.; Tao, O.; Xiao, C.; Lu, C.; Liu, Y. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chem. Cent. J. 2015, 9, 68. [CrossRef]

[5] Liu, Y.; Heying, E.; Tanumihardjo, S.A. History, Global Distribution, and Nutritional Importance of Citrus Fruits. Compr. Rev. Food Sci. Food Saf. 2012, 11, 530–545. [CrossRef]

[6] Turner, T.; Burri, B.J. Potential nutritional benefits of current citrus consumption. Agriculture 2013, 3, 170–187. [CrossRef]

[7] González-Mas, M.C.; Rambla, J.L.; López-Gresa, M.P.; Amparo Blázquez, M.; Granell, A. Volatile compounds in citrus essential oils: A comprehensive review. Front. Plant Sci. 2019, 10, 1–18. [CrossRef]

[8] Mannucci, C.; Calapai, F.; Cardia, L.; Inferrera, G.; D'Arena, G.; Di Pietro, M.; Navarra, M.; Gangemi, S.; Ventura Spagnolo, E.; Calapai, G. Clinical pharmacology of Citrus aurantium and Citrus sinensis for the treatment of anxiety. Evid. Based Complement. Altern. Med. 2018, 2018, 3624094. [CrossRef]

[9] Suntar, I.; Khan, H.; Patel, S.; Celano, R.; Rastrelli, L. An overview on Citrus aurantium L.: Its functions as food ingredient and therapeutic agent. Oxid. Med. Cell. Longev. 2018, 2018, 7864269. [CrossRef]

[10] Li, M.; Liu, L.; Xi, N.; Wang, Y.; Dong, Z. and Xiao, X. (2013). Atomic force microscopy imaging of live mammalian cells. Sci. China Life Sci., 56: 811–817.

[11] Tounsi, M.S.; Wannes, W.A.; Ouerghemmi, I.; Jegham, S.; Njima, Y.B.; Hamdaoui, G.; Zemni, H.; Marzouk, B. Juice components and antioxidant capacity of four Tunisian Citrus varieties. J. Sci. Food Agric. 2011, 91, 142–151. [CrossRef]

[12] Jabri Karoui, I.; Marzouk, B. Characterization of bioactive compounds in Tunisian bitter orange (Citrus aurantium L.) peel and juice and determination of their antioxidant activities. BioMed Res. Int. 2013, 2013, 345415. [CrossRef]

[13] Haraoui, N.; Allem, R.; Chaouche, T.M.; Belouazni, A. In-vitro antioxidant and antimicrobial activities of some varieties citrus grown in Algeria. Adv. Tradit. Med. 2020, 20, 23–34. [CrossRef]

[14] Sarrou, E.; Chatzopoulou, P.; Dimassi-Theriou, K.; Therios, I. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of Citrus aurantium L. growing in Greece. Molecules 2013, 18, 10639–10647. [CrossRef] [PubMed]

[15] Labadie, C.; Ginies, C.; Guinebretiere, M.H.; Renard, C.M.G.C.; Cerutti, C.; Carlin, F. Hydrosols of orange blossom (Citrus aurantium), and rose flower (Rosa damascena and Rosa centifolia) support the growth of a heterogeneous spoilage microbiota. Food Res. Int. 2015, 76, 576–586. [CrossRef] [PubMed]

[16] Deˇgirmenci, H.; Erkurt, H. Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of Citrus aurantium L. flowers. J. Infect. Public Health 2020, 13, 58–67. [CrossRef] [PubMed]

[17] Vopyan,V.G.(1984).Agricultural chemistry. English translation , Mir puplishers .1st edn

[18] Herbert,D. P.; Phillips, J. and Stang, R. E. (1971). Methods in microbiology, J. R. Nawrris and Robbin D. W. (ed). Acad. Press., 513-chap. 3. London. New York

[19] Shameli, K.; Bin Ahmad, M.; Jazayeri ,S.D.; Shabanzadeh, P.; Sangpour,P.; Jahangirian, H. and Yadollah, G. (2012).Investigation of antibacterial properties silver nanoparticles prepared via green method. Chemistry Central Journal, 6:73.

[20] Abou Baker, D.H.; Ibrahim, B.M.M.; Hassan, N.S.; Yousuf, A.F.; Gengaihi, S. El Exploiting Citrus aurantium seeds and their secondary metabolites in the management of Alzheimer disease. Toxicol. Rep. 2020, 7, 723–729. [CrossRef]

[21] Wiley, B. J.; Im, S.; Mc-Lellan, J.; Siekkinen, A. and Xia, Y. (2006) Measuring the surface Plasmon resonance of silver nanostructure through shape controlled synthesis. J. Phys. Chem., B110: 15666.

[22] Falcinelli, B.; Famiani, F.; Paoletti, A.; D'egidio, S.; Stagnari, F.; Galieni, A.; Benincasa, P. Phenolic compounds and antioxidant activity of sprouts from seeds of Citrus species. Agriculture 2020, 10, 33. [CrossRef]

[23] Dandekar, D.V.; Jayaprakasha, G.K.; Patil, B.S. Hydrotropic extraction of bioactive limonin from sour orange (Citrus aurantium L.) seeds. Food Chem. 2008, 109, 515–520. [CrossRef] Molecules 2021, 26, 5832 15 of 18

[24] Khettal, B.; Kadri, N.; Tighilet, K.; Adjebli, A.; Dahmoune, F.; Maiza-Benabdeslam, F. Phenolic compounds from Citrus leaves: Antioxidant activity and enzymatic browning inhibition. J. Complement. Integr. Med. 2017, 14, 1–13. [CrossRef] [PubMed]

[25] Suryawanshi, J.A.S. An overview of Citrus aurantium used in treatment of various diseases. Afr. J. Plant Sci. 2011, 5, 390–395.

[26] Isabel, M.; Carvalho, F.; Osta, M. Anxiolytic and Sedative Effects of Extracts and Essential Oil from Citrus aurantium L. Pharm. Soc. Jpn. 2002, 25, 1629–1633.

[27] Gholivand, M.B.; Piryaei, M.; Abolghasemi, M.M. Analysis of volatile oil composition of Citrus aurantium L. by microwave- assisted extraction coupled to headspace solid-phase microextraction with nanoporous based fibers. J. Sep. Sci. 2013, 36, 872–877.

[28] Periyanayagam, K.; Dhanalakshmi, S.; Karthikeyan, V.; Jagadeesan, M. Phytochemical studies and GC/MS analysis on the isolated essential oil from the leaves of Citrus aurantium Linn. J. Nat. Prod. Plant Resour. 2013, 3, 19–23. alcohol: the ESCALE study (SFCE). Cancer. 2008.