



Green Synthesis of Nanoparticles Using Aqueous Leaves extract of *Parkinsonia aculeata* L. and Evaluation Their Resistance to Salt Stress of Some Aquatic Plants

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Abstract:

Salinity is one of the main abiotic factors that has an impact on a plant's development and growth. The importance of biosynthesis silver nanoparticles from the *Parkinsonia aculeata* L. plant due to its unique physical and chemical properties and large surface area relative to its size. AgNPs were characterized using UV-Vis, FT-IR, XRD, SEM and TEM techniques , UV-Vis showed that the top absorption of AgNPs was at 420 nm wavelength, confirming the presence of silver nanoparticles , Use the FT-IR device to confirm the formation of AgNPs through bioactive functional groups and reaction sites found in the plant extract that were shown as a reduction agent to create AgNPs , And the XRD test showed that the AgNP size rate was 38.45nm , The FE-SEM showed that AgNPs were spherical in shape and size ranging from 30.21 nm to 64.03nm . In this study we used biosynthesis silver nanoparticles to help *Cratophyllum demersum* grow in the aquatic environment under salt stress conditions. It was used in 3 concentration (0.05, 0.1, 0.15 g) to understand its impact on the aquatic environment and the carrying of aquatic plants for salt stress. As a result of this study, the application of these nano-silver particles has improved the growth rate and the amount of chlorophyll and TOC and has made the properties of the aquatic environment move in a positive direction and also reduced the proline content. So, we conclude that AgNPs is a cheap and effective application for environmental treatment, especially water treatment.

Keywords: AgNPs, Salinity, Plant Extracts, Water physicochemical parameter, *Ceratophyllum demersum*, *Parkinsonia aculeata* L.

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Introduction

One of the main abiotic factors that has an impact on a plant's growth and development is salinity (Gharsallah *et al.*, 2016). As a result of ionic imbalance and osmotic stress caused by high salt levels, plants suffer severe effects on their morphology, biomass, and metabolic activities, which ultimately lead to plant destruction (Zhang *et al.*, 2013; Rahnesan *et al.*, 2018). High salt concentrations—typically sodium chloride—

cause ionic stress by specifically inhibiting metabolic activities, which in turn causes osmotic stress by lowering the water potential within cells (Di Martino *et al.*, 2003). Saline water can significantly affect plant physiological and biochemical responses, including general disturbance of the nutritional status of plants, osmotic stress, and ion-specific toxicity (Nazar *et al.*, 2011). In many countries, the dry climate is

the principal contributor to the salt of the water (Bsoul *et al.*, 2017).

Aquatic life is subject to a number of abiotic stresses, particularly freshwater salinity, which can affect an organism's traits in a number of ways (such as its ability to survive, reproduce, photosynthesize, grow, etc.) (Canedo-Arguelles *et al.*, 2013).

Therefore, there is a constant need to create innovative methods to mitigate Nanoparticles, also known as Nano Scale Particles, are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Ball, 2002; Roco, 2003). Compared to the bulk material, nanoparticles have significantly different physicochemical properties (Nel *et al.*, 2006). Since they have a unique combination of physical, chemical, and biological qualities and have a huge surface area compared to their tiny size (Nanometer), nanoparticles are a subject of great attention. The stability, anti-bacterial, sterility, light absorption, and dispersion of nano-silver particles are some of their most significant characteristics. (Ealias and Saravanakumar, 2017).

The aim of this study uses a modern and ecofriendly method of nanotechnology (the silver

nanoparticles biosynthesis from *P. aculeata*) to reduce the impact of salt stress on *C. demersum* and to improve the properties of the aquatic environment and to know its positive impact on this plant

Materials and Methods

Plant sample collection:

P. aculeata L. was collected from the Abu Al-Khasaib region during July, August and November 2022.

Preparation of the aqueous extract of *Parkinsonia aculeata* L.

According to a method (Manokari *et al.*, 2016), the leaves of a *P. aculeata* L. were thoroughly washed in distilled water to remove contaminants from the surface, dried in an oven at 105°C for an hour, and then ground into 25 g before being added to a 500 ml glass bowl with 150 ml of deionated distilled water. The mixture was then heated using the magnetic bar continuously for 30 minutes. The extract was subsequently nominated using a Whatman filter and stored at 4°C in the fridge for later use.



Figure 1. aqueous extract of *P. aculeata* L.

Preparation of AgNO₃ solution:

A solution of silver nitrate with a 1 mM concentration was made by dissolving 0.016 g of silver nitrate in 100 ml of deionated distilled water.

Biosynthesis of silver nanoparticles (green synthesis)

According to the protocol (AL-Othman *et al.*, 2017), 100 mL of a 1 mM AgNO₃ solution was added in a ratio of 1:10 to 10 ml of *Capparis spinosa* L.'s aqueous extract to create AgNPs. The mixture was heated at 60 °C until the color of the mixture changed from orange yellow to dark brown, signifying the beginning of the creation of AgNPs.

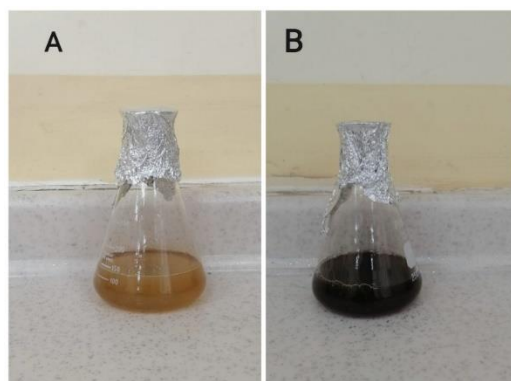


Figure 2. The final coloring of the aquatic extract of the *P. aculeata* after adding it to the silver nitrate and forming the nano-silver particles.

A: Color change 30 minutes after the reaction

B: Color change 48 hours after the reaction.

Characterization of Green Synthesized Silver Nanoparticles

Application of UV-Vis's spectroscopy, a potent analytical instrument, was employed to evaluate the environmentally friendly AgNPs and validate the reduction process for AgNP production. Fourier transform infrared spectroscopy was performed using a Thermo Scientific Nicolet 6700 FT-IR spectrometer (Waltham, MA, USA) to look for potential biomolecules and functional groups. The structure of the created silver nanoparticles was examined by X-ray diffraction (Rigaku Ultima IV, Neu-Isenburg, Germany XRD). Two ranges were used to collect the data. The crystallite domain size was determined using D. Scherrer's equation. Images of the particle morphology and size of the phyto-mediated AgNPs were captured using transmission electron microscopy (TEM; JEM-1011; JEOL Ltd., Tokyo, Japan).

Description of experience

C. demersum was collected from the Qurnah region, taking into account that it was close to age and was washed well to remove the suspended contaminants and the remains of the living, and then placed in the tubs of its retrofit before experimenting. The experiment was conducted in the laboratories of the Department of Environmental Science at the College of Sciences

for 4 weeks to determine the success of biosynthesis silver nanoparticels from *P. aculeata* in improving the various aquatic environmental properties, resisting salt stress and also studying its impact on the plant *C. demersum*.

It weighs 20 grams of plant *C. demersum* was placed in plastic basins plus 5 litres of water taken from the area near Najibiya, and the experiment consisted of 4 basins (3 treatment basins with 3 concentrations of AgNPs (0.05, 0.1, 0.15 g) and a control basin

Measurement of physical and chemical properties of water:

- 1- Total soluble solids, electrical conductivity and temperature were measured using HANA HI9813-6. Total soluble solids, electrical conductivity, and temperature were expressed as in ppm, $\mu\text{S}/\text{cm}$, and Celsius, respectively.
- 2- Salinity was calculated as the product times the electrical conductivity values of 0.64 and expressed as a unit mg/L.
- 3- The pH-meter device was calibrated with standard solutions before measuring hydrogen ions.
- 4- The dissolved oxygen in the water was measured using the water dissolved oxygen meter type Extech and the results was expressed in a mg/l unit.

Measurement of plant characteristics:**Measurement of relative growth rate**

The equation provided in Xiaom *et al.* 2004 was used to calculate the relative growth rate of plants.

Growth rate % = final soft plant weight in g / first soft plant weight in g

Measurement of proline

According to the Trollant and Lindsley method (1955), took 0.2 grams of plant leaves of *C. demersum*, added 5 ml of ethyl alcohol at 95% concentration, then carried out a centralized package for 30 minutes, dried up with air, added 2 millimeters of distilled water, and then carried out a central discharge again, and then took 1 ml, and the absorption was read using the spectrometer and at the 520 nm wavelength, then estimated the sample content of the proline.

measurement the chlorophyll a, b, total, carotene. In accordance with the method described in Arnon, (1949), 1 grams of *C. demersum* leaves, was to added 20 ml of acetone 80%, stored the sample for 24 hours, and crushed by ceramic mortar to get the green material of plant, the mixture was filtered and the filtrate was used to measure the chlorophyll a at 663 nm and chlorophyll b at 645 nm using the spectrophotometer using equations.

Chlorophyll a (mg/g) = $[12.7(A_{663}) - 2.69(A_{645})] * V / 1000 * W$ (1)

Chlorophyll b (mg/g) = $[22.9 (A_{645}) - 4.68(A_{663})] * V / 1000 * W$ (2)

Total Chlorophyll (mg/g) = $[20.2(A_{645}) + 8.02 (A_{663})] * V / 1000 * W$ (3)

In the same way, the concentration of the Carotenoid on wavelength was measured 480 nm according to Kirk and Allen, 1965, according to the following equation:

Carotenoid (mg/g) = $[A_{480} + (0.114 * A_{663}) - (0.638 * A_{645})] * V / 1000 * W$ (4)

Measurement of total organic carbon.

According to Ball, 1964, organic carbon was determined by placing 0.5 g of dry plant material in a concrete cranium with a specified weight, putting the cranium in a Furnace oven at 550 C° for three hours, and then weighed. TOC% was calculated according to equation:

Total organic carbon %TOC = the weight of the burrow before burn - the weight of the burrow after burn (1) \

Gas chromatography - mass spectrometer:

Effective compounds were calculated in the aqueous extract of the plant *P. aculeata* (Haarbom, 1984) at the Basrah Oil Company's, Nehran Ben Omar laboratories using the GC-MS device manufactured by Shimadzu.

Statistical Analyses:

The mean separation between treatments was calculated using Duncan's new multiple range test (p 0.05) and two-way ANOVA in SPSS version 20 for Windows.

Results and Discussion**Characterization of AgNPs Nanoparticles****UV-Visible Spectrum**

After 1 mM AgNO₃ was added, the *P. aculeata* leaf extract's color changed from orange-yellow to dark brown in the reaction mixture with AgNO₃ solution. It's possible that this color change is a precursor to the creation of AgNPs. However, but in order to do additional research, The surface plasmon resonance (SPR) of the silver ion was discovered using a UV-Vis spectrophotometer. Due to the plasma surface absorption phenomena, the greatest absorption point at wavelength is 420 nm, and this is for a peak within the diagnostic limits of nano-silver particles, which range from 400 to 450 nm (Alwaan *et al.*, 2021) as in shape.

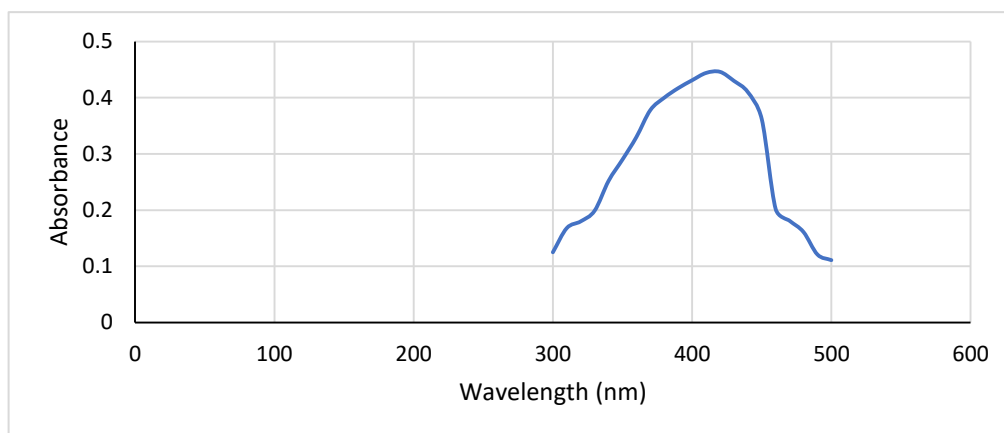


Figure 3. UV-visible spectrum of the silver nanoparticles (AgNPs)

FT-IR Spectrum

The functional groups employed in the production of AgNPs were found by FTIR spectroscopy, where the FT-IR profile demonstrated seven peaks located at about 3456, 3300, 2924, 1647, 1523, 1238 and 1041 cm^{-1} . In the process of creating AgNPs, phenol groups function as significant reducing agents; this was confirmed through package 3300 cm^{-1} which was responsible for stretching vibration of the O-H extension of carboxyl acid and phenol (Fritsch *et al.*, 2021). the peak 3456 cm^{-1}

referred to insistent N-H for the aides. The 2924 cm^{-1} peak referred to stretching vibration of aliphatic C-H, the peak at 1647 cm^{-1} as well as 1523 cm^{-1} as a attributed to vibration of Aromatic C=C (Kargar *et al.*, 2021; Ali *et al.*, 2023). Finally, packages 1238 cm^{-1} and 1041 cm^{-1} referred to C-N and C-O of aromatic Amine groups and organic acid or alcohol (Jasim *et al.*, 2023). The apparent bands are representative of functional groups of various compounds as in Figure 4.

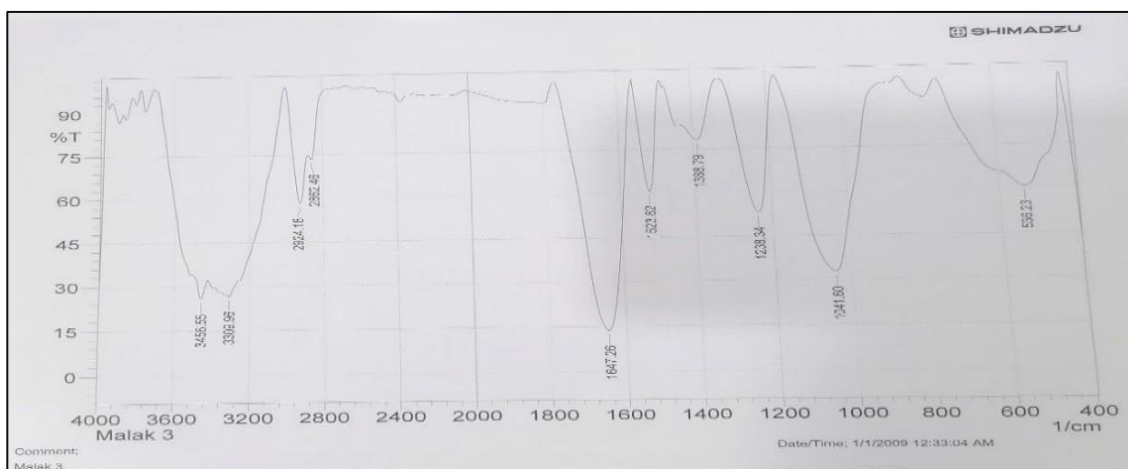


Figure 4. FT-IR spectrum of synthesized AgNPs

X-ray Diffraction analysis

AgNPs' size and crystal nature were revealed using an X-ray Diffraction spectrometer. Using

the Debbye Scherrer equation, the AgNPs crystal size rate was determined at 100% peak, and the crystal volume rate was 38.45 nm

Table (1) X-ray spectrometer data for nano-silver particles synthesis from *P. aculeata*

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
10.4359	4.14	0.4723	8.47704	14.41
27.8654	10.85	0.2362	3.20180	37.75
32.2283	28.73	0.2362	2.77763	100.00
38.1769	6.21	0.7872	2.35741	21.60
46.2207	15.26	0.3149	1.96415	53.09
57.5010	3.41	0.4723	1.60279	11.86
76.0067	3.68	0.1574	1.25212	12.82
77.1654	0.79	1.9200	1.23516	2.75

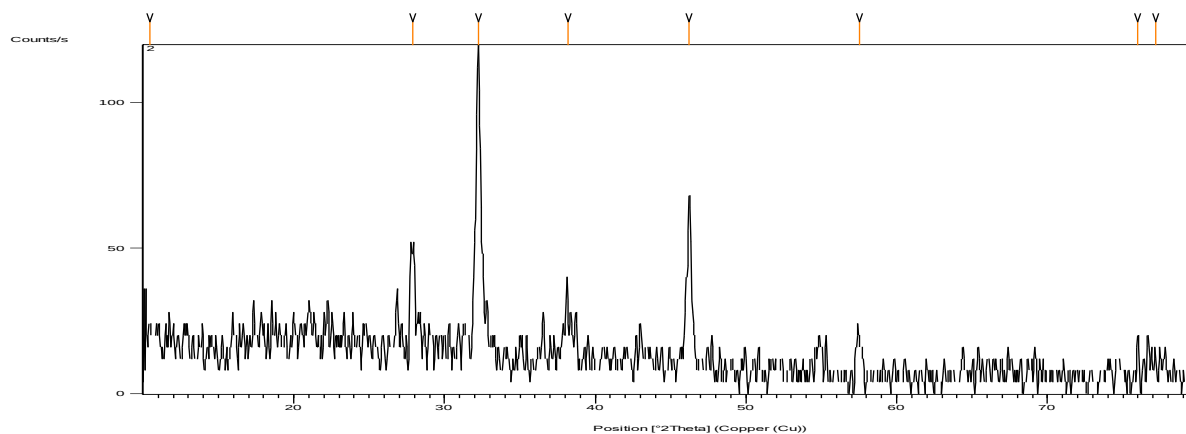


Figure 5. XRD patterns

SEM analysis

Figure 6 displayed a SEM image of synthesized AgNPs. According to the SEM image, the generated AgNPs were spherical and had a narrow size range between 30.21 and 64.03 nm. The scanning electron microscope (SEM)

revealed spherically shaped, differently sized silver nanoparticles with an AgNPs size rate of 41.34 nm. If the particles created are spherical, then this result is compatible with Shaikhaldein *et al.*, 2022.

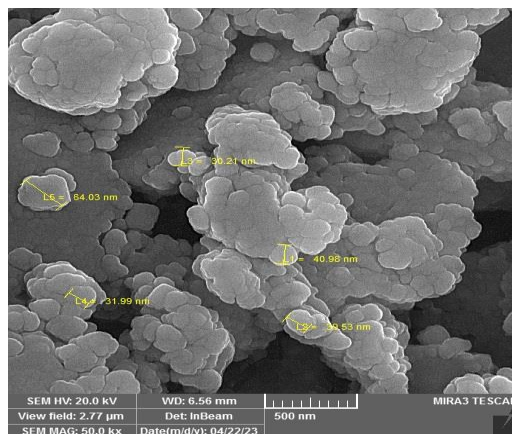


Figure 6. SEM image of synthesized AgNPs

Physiochemical properties of water

Dissolved solids

Figure 7 showed the values of dissolved solids in AgNPs and biosynthesis from *P. aculeata* in all concentrations (0.05, 0.1, 0.15) g were declining compared to the control sample. At the end of the study period, soluble solids in the control basin rose to 2730 ppm, and in the AgNPs treated basins decreased to (2270, 2070

and 1570) ppm for concentrations (0.05, 0.1, 0.15) g.

The increase in ions and substances from plant metabolic processes is the cause of the increase in dissolved solids at both the beginning and end of the experiment, and the decrease in treatment basins is due to the absorption of these substances on the surfaces of these AgNPs .

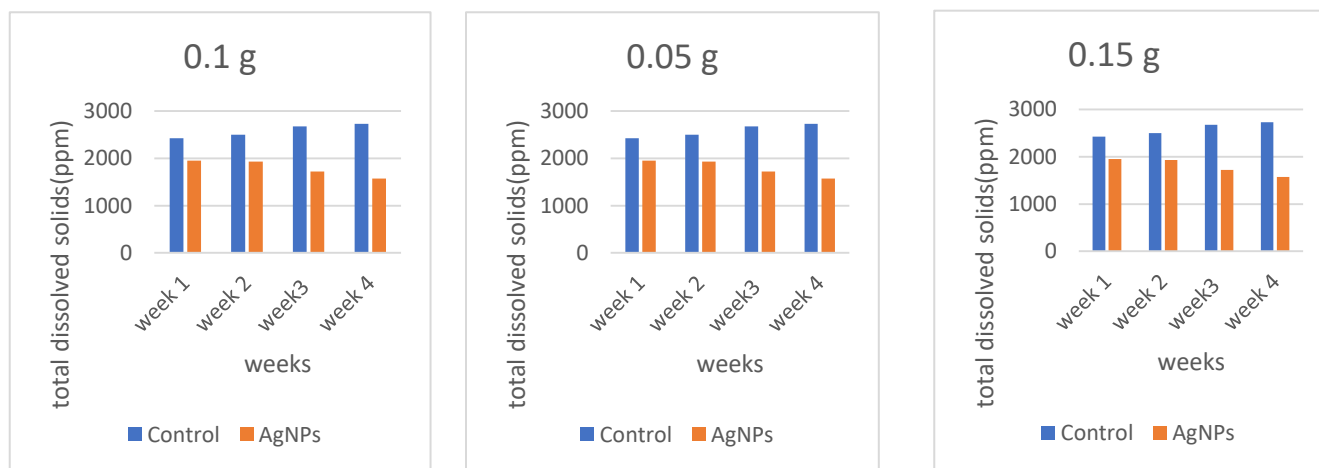


Figure 7. Impact of the change in AgNPs on the values of dissolved solids

EC

The study showed that the values of EC in AgNPs biosynthesis from *P. aculeata* in all concentrations (0.05, 0.1, 0.15) g were declining compared to the control sample. At the end of the study period, EC in the control basin rose to 5264 $\mu\text{S}/\text{cm}$, and in the AgNPs treated basins

decreased to (4738, 4610, 2570) $\mu\text{S}/\text{cm}$ for concentrations (0.05, 0.1, 0.15) g-. Electrical conductivity is one of the most important measures of the amount of salt melted into the water (Al-Hmoody, 2022). stress generated by sodium chloride ion led EC to rise in the control basins, whereas *P. aculeata* biosynthetic

nanoparticles' ability to absorb dissolved ions on their surface caused EC to drop in the treated

basins, as shown in Figure 8.

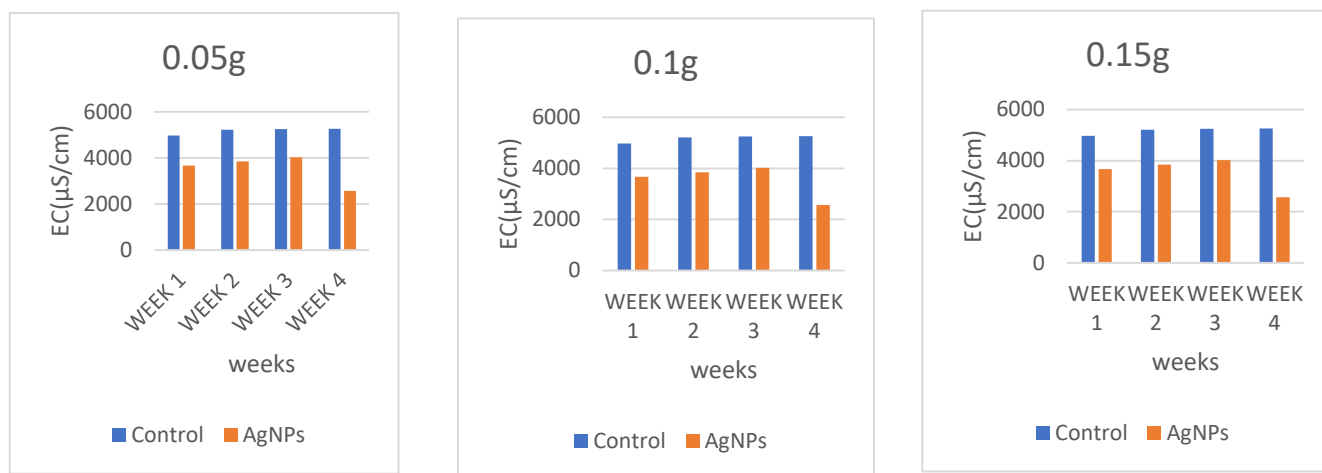


Figure 8. Impact of the change in AgNPs on the values of EC during (0.05, 0.1 ,0.15) g

Salinity

The study showed that the salinity values in the biosynthesis of AgNPs from *P. aculeata* reduced at all dosages (0.05, 0.1, and 0.15) g in comparison to the control sample. Salinity in the control basin rose to 3368.9 mg/L at the end of the trial, whereas it dropped to (3032.3, 2950.4, 1644.8) mg/L for concentrations (0.05, 0.1, 0.15g) in basins treated with AgNPs, as shown in Figure 9. Salinity is an important element of the aquatic environment that affects the dispersal and growth of submerged plants, which in turn affects the survival of aquatic plants (Shakweer, 1993). The reduction in salinity in basins treated

with AgNPs is brought about by the adsorption of salt ions on the surface of these particles. Shaikhaldein *et al.* (2022) claim that salt stress reduces oblongifolia M evolution, which in turn decreases plant development. Aquatic plants grow faster, produce more biomass, and are better able to withstand salt stress due to the application of AgNPs. According to (Shafi *et al.*, 2010) and (Hussain *et al.*, 2015), the plant's poor growth is probably due to the plant's inability to absorb adequate nutrients or the increased absorption of sodium ions.

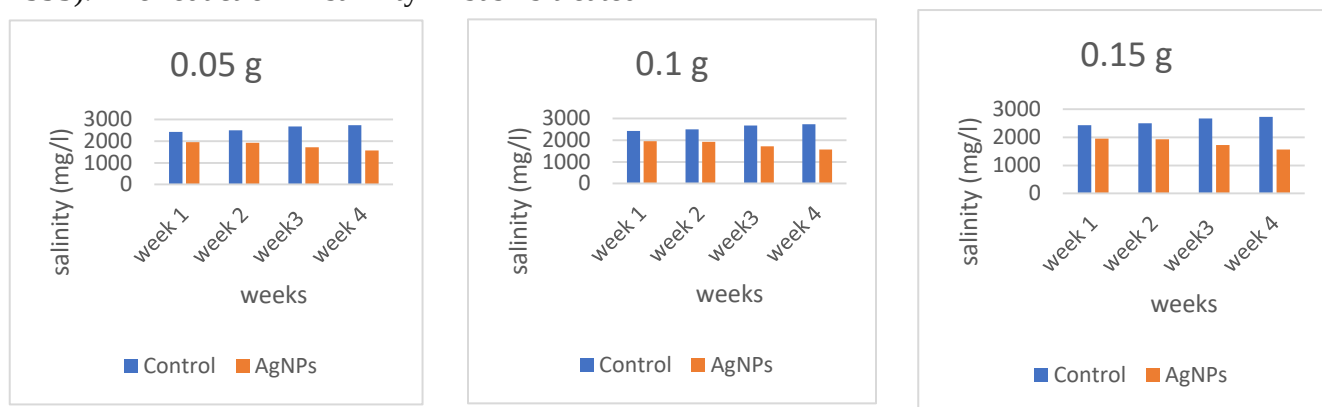


Figure 9. Impact of the change in AgNPs on the values of salinity during (0.05, 0.1 ,0.15) g

pH

Figure 10 displayed that the higher pH value in the AgNPs basin compared to the control basin. The lower pH in the control basin may be brought on by sodium chloride ions, which would result in less photosynthesis and an acidic environment. In contrast, the higher pH value in the AgNPs basin is a result of the consumption

of CO₂ gas during the photosynthesis process by aquatic plants, which turns the pH value towards the base (Van der valk, 2012; Li *et al.*, 2015). and most organisms are sensitive to any change in pH, because it affects the chemo-reactors of water (Al-Saddy, 2018).

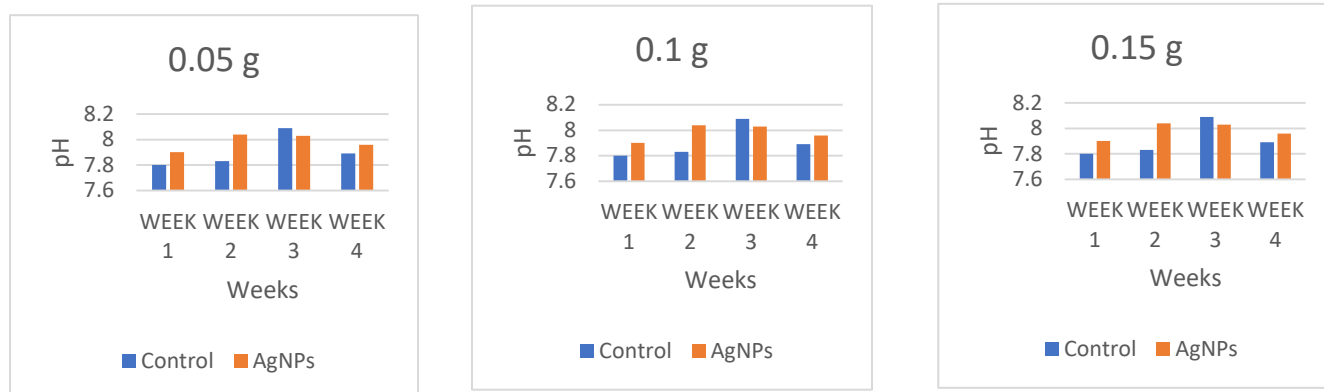


Figure 10. Impact of the change in AgNPs on the values of pH during (0.05 , 0.1 ,0.15) g

The dissolved oxygen (DO)

As seen in Figure 11, the results of this study showed that dissolved oxygen values where it was close within weeks 1 and 2 of the experiment, while the third week was control basin more than the AgNPs, and the fourth week was also close. Living requires dissolved

oxygen, according to (Durmishi *et al.*,2008). In the basins, the mortality of submerged plants caused a drop in the photosynthesis process, which is a source of dissolved oxygen, but the aquatic plants' illumination is what causes it to be at its highest.

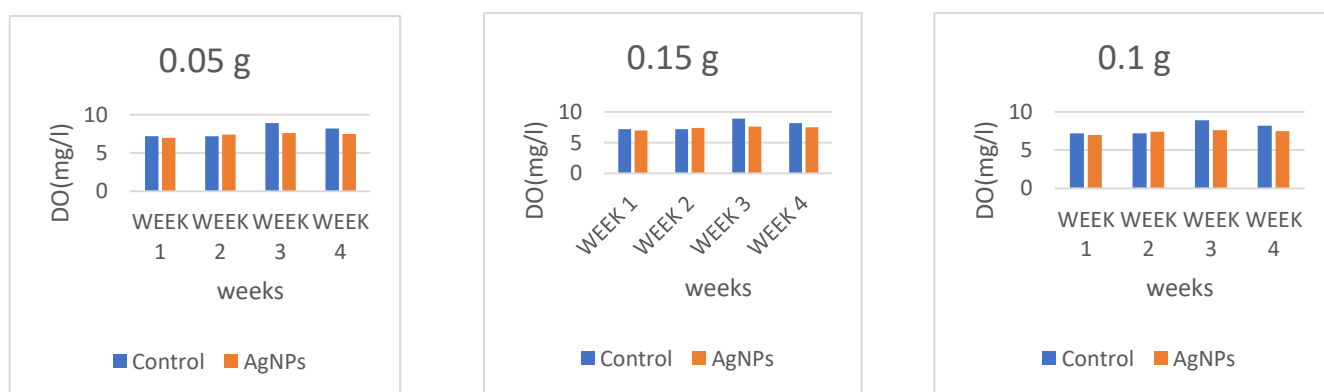


Figure 11. Impact of the change in AgNPs on the values of DO during (0.05 ,0.1 ,0.15) g

Measurement of plant characteristics

Measurement of relative growth rate

The study showed that the relative rate of *C. demersum* plants was higher in AgNPs treated basins from the control basin, with the lowest

relative growth rate of 0.825% in the control basin and the highest value of 1.139% at concentration 0.1g as the Figur. The potential of AgNPs to enhance plant development, increase living mass, and improve plant tolerance for salt

stress, which he has proved, may be the cause of the high growth rate (Nejatzadeh, 2021;

Shaikhaldein *et al.*, 2022).

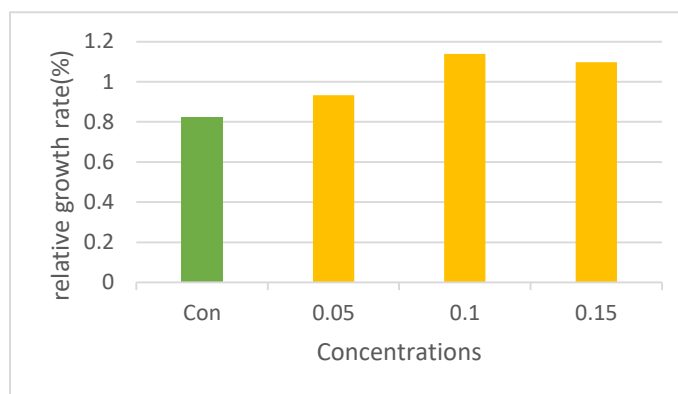
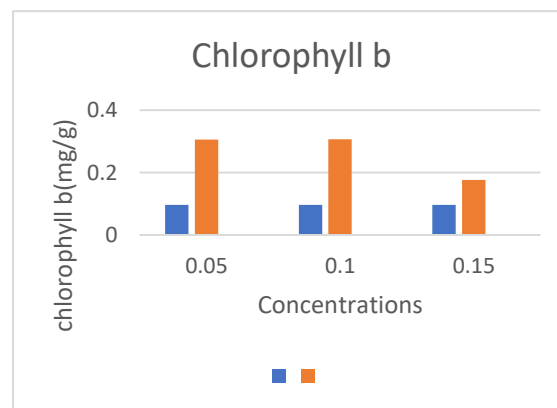
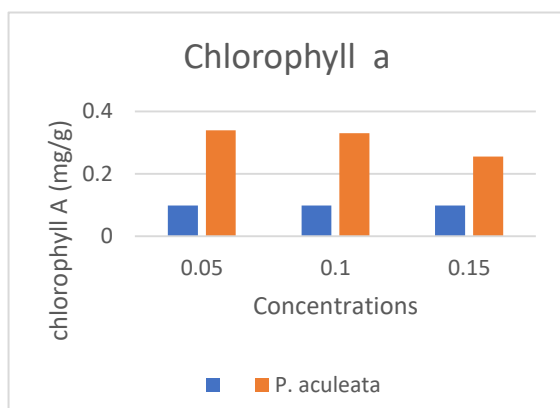


Figure 12. Impact of the change in AgNPs on the relative growth rate

Measurement The chlorophyll a, b, total, carotene.

The results showed that treated basins with AgNPs had greater values of chlorophyll a, b, c, and total than untreated basins. as seen in Figure 13, were the highest value of chlorophyll a 0.34 mg/g at 0.05g in the AgNPs treated basins and the lowest value of 0.0986 mg/g in the control basin. While, the highest value of chlorophyll b 0.307 mg/g at 0.1g in the AgNPs treated basins and the lowest value of 0.097 mg/g in control basin. the highest value of chlorophyll total 0.677 mg/g at 0.1g in the AgNPs treated basins and the lowest value of 0.195 mg/g and also the highest value of carotenoid 0.01 mg/g at 0.05g in the AgNPs treated basin, and the lowest value 0.0056 mg/g in the AgNPs treated basins at 0.15g and control basin. The *C. demersum* in AgNPs basins contained more chlorophyll than

the control sample, according to the study's findings. Low chlorophyll in plant leaves is the most important biological indicator of plant stress (Mahdi, 2018). According to (Wu, 2015), salt stress, which also hurts green polymers, is the cause of the drop in chlorophyll in the control basin. Salt stress also prevents the body from absorbing nutrients needed for the formation of the chlorophyll molecule. The increase in chlorophyll in treated basins is due to the application of AgNPs, which efficiently enhances chlorophyll dye under salt stress conditions. Because AgNPs have expedited growth by increasing chlorophyll levels and improving optical system performance, this result is consistent with (Shaikhaldein *et al.*, 2022).



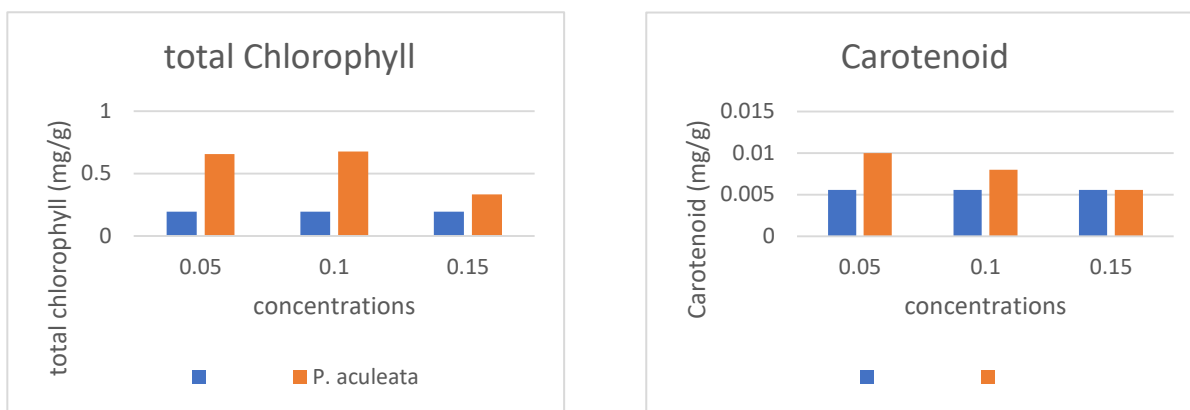


Figure 13. The effect of the change in AgNPs on the amount of chlorophyll A, B, total and carotenoid Measurement of proline

The figure 14 shows the low proline content of *C. demersum* with the increased amounts added from AgNPs, the highest amount of proline was 0.13 in the basins with silver nanoparticle at 0.05g concentration and lesser 0.018 in the basin with AgNPs at 0.15g, compared to the control sample that has proline 0.212. The *C. demersum* plant in the control basin may have a

high proline content because of salt stress. Because plants produce more proline in their cytosol to combat the toxic effects of sodium chloride, which results in extra osmosis, adding AgNPs to the water will be crucial in assisting plants in lowering their proline production., which results are similar to (Shaikhaldein *et al.*, 2022).

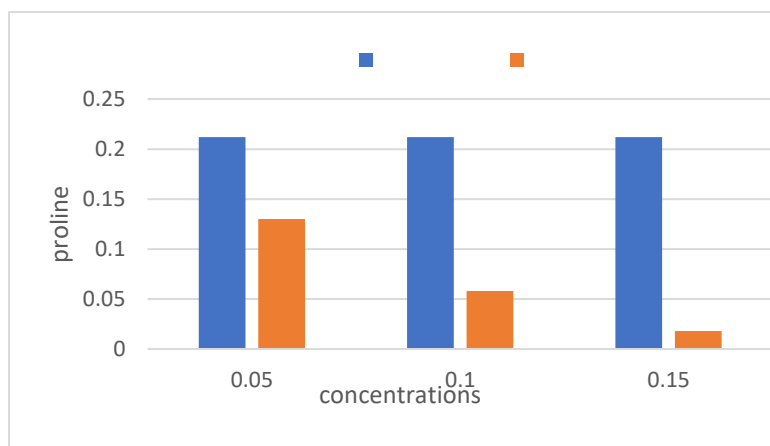


Figure 14. Impact of the change in plant extract concentrations and AgNPs on proline content

Measurement of total organic carbon

The results of this study showed an increase in the total organic carbon at the end of the experiment and in all basins treated with AgNPs compared to the control sample, the highest organic carbon in the basin treated by AgNPs of

0.21 g and the lowest in the control basin of 0.12 g. The large rise may be due to the *C. demersum* plant's increased biomass as a result of improved photosynthesis brought on by AgNP processing, which lessens salt stress and enhances light construction, as in the Figure 15.

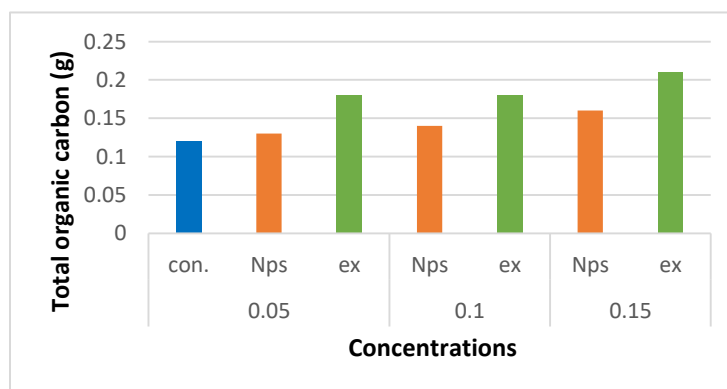


Figure 15 . The effect of the change in AgNPs on the total organic carbon

Conclusions

The study showed that bio-manufactured silver particles of *P. aculeata* have an active and significant role in improving the properties of the aquatic environment, increasing the capacity of the plant to resist salt stress and thus improving the characteristics of the plant. The application of this technology helps aquatic plants to grow under salt stress conditions, and the use of nano-silver particles is economically beneficial, as they are cheap and have no negative side effects.

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التخليق الأخضر للجسيمات النانوية باستعمال مستخلص *Parkinsonia aculeata* L وتقييم مقاومتها للإجهاد الملحي لبعض النباتات المائية

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الخلاصة :

الملوحة هي أحد العوامل الحيوية الرئيسية التي تؤثر على تنمية النباتات ونموها. أهمية الجسيمات الفضة النانوية المصنعة حيويًا من نبات *P. aculeata* L. بسبب خصائصها الفيزيائية والكيميائية الفريدة ومساحتها السطحية الكبيرة بالنسبة إلى حجمها. وشخصت هذه الجسيمات الفضة النانوية باستخدام تقنيات مطياف الأشعة فوق البنفسجية والمرئية، مطياف الأشعة تحت الحمراء، حيود الأشعة السينية والمجهر الإلكتروني الماسح للانبعاث المجالي، أظهرت الأشعة فوق البنفسجية-المرئية أن قمة الامتصاص لـ AgNPs كان عند طول الموجي 420 نانومتر، مما يؤكد وجود جزيئات فضة نانوية، استخدام جهاز مطياف الأشعة تحت الحمراء للتأكد من تكوين AgNPs من خلال مجموعات وظيفية حيوية نشطة ومواقع التفاعل الموجودة في المستخلصات النباتية التي ظهرت كعامل اختزال لإنشاء جسيمات الفضة النانوية، وأظهر اختبار حيود الأشعة السينية أن معدل حجم جسيمات الفضة النانوية كان 38.45 نانومتر، أظهرت المجهر الإلكتروني الماسح للانبعاث المجالي أن جسيمات الفضة النانوية هي كروية الشكل والحجم تتراوح بين 30.21 نانومتر إلى 64.03 نانومتر. وقد استخدمنا في هذه الدراسة جسيمات الفضة النانوية المصنعة حيويًا لمساعدة نبات الشمبلان *C. demersum* على النمو في البيئة المائية في ظل ظروف إجهاد الملح. وقد استخدمت في 3 تركيزات (0.05، 0.1، 0.15 غرام) لفهم تأثيرها على البيئة المائية وتحمل النباتات المائية للإجهاد الملحي. ونتيجة لهذه الدراسة، أدى تطبيق هذه جسيمات الفضة النانوية إلى تحسين معدل النمو وكمية الكلوروفيل ومحتوى الكربون العضوي الكلي، وجعل خواص البيئة المائية تتحرك في اتجاه إيجابي كما قلل من المحتوى البروتيني. لذلك نستنتج أن جسيمات الفضة النانوية هو تطبيق رخيص وفعال لمعالجة البيئة، وخاصة معالجة المياه.

الكلمات المفتاحية : AgNPs، الملوحة، المستخلصات النباتية، Ceratophyllum، Water physicochemical parameter، *Parkinsonia aculeata* L، *C. demersum*.