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Original Research Article

Physiological Effects of Nanoparticles Prepared from Olive Leaf Extract and Copper Oxide on Strawberry Plants

Alyaa Muhsin Yousif[1*]
[1]College of Science, Mustansiriyah University, Baghdad, Iraq

***Corresponding Author:** Alyaa Muhsin Yousif
College of Science, Mustansiriyah University, Baghdad, Iraq

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Abstract: ***Background***: Nanoparticles, due to their small size and large surface area, have unique physicochemical properties that make them attractive for a wide range of industrial, medical, agricultural application. ***Objective***: To prepare a novel nanoparticles from olive leaf extract and copper oxide and test its physiological effect of strawberry plants. ***Materials and Methods***: Olive leaves extract was prepared according to a certain protocol, and the green nanoparticles were synthesized by coordination this extract with copper oxide. Characterization was done using many techniques such as FTIR, UV-Vis, XRD to identify the new copper nanoparticles. Different concentrations were prepared of CuONPs (10%, 20%, and 30%). ***Results***: The FTIR spectrum shows peaks which confirm the coordination of olive leaves extract with the copper ion, while UV-Vis spectrum shows peaksthat confirm the formation of copper nanoparticles, the SEM shows the homogeneity of the nanoparticle that was confirmed by XRD spectrum and dataUsing30% (C3) give best seedling leaf length, and number of fruitswhile number of leaves are best in 20% (C2) compared to other concentrations. While the highest value in the number of flowers in treatmentof bio fertilizer. Best concentration of DNA appears in C3 (134.7) while best DNA purity is found on C2 (20%). The chlorophyll was also measured using SPADwhich show optimum with C3 (30%). ***Conclusions***: Using30% (C3) give best seedling leaf length, number of fruits and chlorophyll while number of leaves are best in 20% (C2).

Keywords: Olive Leaves Extract, Copper Oxide, Green Nanoparticles, Strawberry Plant.

INTRODUCTION

Nanoparticles, due to their small size and large surface area, have unique physicochemical properties that make them attractive for a wide range of industrial, medical, and consumer applications. However, the small size of nanoparticles also means that they can interact with biological systems in ways that larger particles cannot [1, 2].

In 2013, a study was carried out by Ambalavanan and his coworkers [3], the study shows that some nanoparticles, particularly those made of metals like titanium dioxide and silver, have been shown to induce inflammation in the lungs and other organs. This can lead to respiratory distress and other health problems [3], while (Abdal Dayem *et al.*, 2017) depicted that nanoparticles can generate reactive oxygen species (ROS), which can cause oxidative stress in cells. This can damage cell membranes, DNA, and proteins, leading to a range of adverse effects [4], however in the same year, (Behzadi *et al.*, 2017) shows that nanoparticles can be taken up by cells through a variety of mechanisms, including endocytosis and phagocytosis. Once inside the cell, nanoparticles can interact with organelles and disrupt cellular processes [5- 7].

It is worth noting that the physiological effects of nanoparticles can vary depending on their size, shape, surface chemistry, and other properties. Therefore, it is important to carefully evaluate the potential risks associated with specific types of nanoparticles before they are used in consumer products or medical applications [2].

Nanoparticles prepared from olive leaf extract and copper oxide are a type of nanomaterial that is synthesized through a green chemistry approach. Olive leaf extract is used as a reducing and capping agent for the formation of copper oxide nanoparticles, which possess various unique physical, chemical, and biological properties [8, 9].

Nanoparticles Prepared From Olive Leaf Extract And Copper Oxide Has Been Studied For Their Potential Effects On Strawberry Plants. Here Are Some Of The Reported Effects:

- Growth promotion: Studies have shown that the application of nanoparticles prepared from olive leaf extract and copper oxide can promote the growth of strawberry plants. This includes an increase in plant height, stem diameter, and number of leaves [10].
- Improved nutrient uptake: Nanoparticles can improve the uptake of nutrients by plant roots. This includes an increase in the uptake of nitrogen, phosphorus, and potassium, which can lead to improved plant growth and yield [11].
- Increased antioxidant activity: The nanoparticles can also increase the antioxidant activity of strawberry plants. This includes an increase in the levels of enzymes such as superoxide dismutase, catalase, and peroxidase, which help to protect the plants from oxidative stress [12].
- Resistance to pathogens: Nanoparticles prepared from olive leaf extract and copper oxide has been shown to have antifungal and antibacterial properties. This can help to protect strawberry plants from common pathogens, such as Botrytis cinerea and Colletotrichum acutatum, which can cause diseases like gray mold and anthracnose [13].

The Current Study Is To Investigate The Effect Of Nanoparticles Prepared From Olive Oil And Copper Oxide On Strawberry Plants.

MATERIALS AND METHODS

Preparation of Botanical Extract from Olive Leaves

Choose healthy olive leaves from mature olive trees. It's best to gather the leaves during the morning when their essential oil content is highest. Then, thoroughly wash the leaves with clean water to remove any dirt or debris, after that allow the leaves to air dry in a well-ventilated area until they become crisp, around 40-50°C is used to speed up the drying process. Make sure the leaves are completely dry to prevent mold or bacterial growth in the extract.Once the leaves are dry, grind them into a fine powder using a mortar and pestle or a grinder. Then infusion method was used through adding the powdered olive leaves and pour hot water over them (not boiling). The ratio of olive leaves to water is typically 1:10 (e.g., 10 grams of leaves for every 100 milliliters of water). Cover the container and let it steep for 15-30 minutes. Afterward, strain the liquid to separate the extract from the plant material. Filtration must be carried out to remove any remaining sediment or impurities, and the extract to a sterilized, airtight glass container, preferably dark-colored to protect it from light and store it in a cool, dry place away from direct sunlight.

Synthesis of Copper Oxide Nanoparticles

Preparation of copper precursor solution: Dissolve a copper salt, such as copper chloride (CuCl2) or copper nitrate (Cu (NO3)2), in distilled water to prepare a copper precursor solution. The concentration of the solution can vary depending on the desired nanoparticle size and concentration, and then add a base, such as sodium hydroxide (NaOH) or ammonium hydroxide (NH4OH), to the copper precursor solution while stirring continuously. The base reacts with the copper ions to form copper hydroxide, the copper oxide precipitate needs to be washed to remove impurities and excess reactants. The collected copper oxide nanoparticles are usually washed with a suitable solvent, such as ethanol or acetone, to remove residual impurities. Afterward, the nanoparticles are dried using techniques like vacuum drying or oven drying.

The Preparation of Olive Copper Nanoparticles

Once both the olive leaf extract and the copper oxide nanoparticles were completed, proceeding to prepare the nanoparticles is carried out, by which the olive leaf extract is used as a reducing and capping agent for the synthesis of copper oxide nanoparticles. This involves adding the olive leaf extract to a copper oxide nanoparticle suspension and allowing the reaction to occur under suitable conditions (e.g., temperature and stirring) to promote the reduction of copper ions on the nanoparticle surface by the bioactive compounds in the extract.

Characterization of Olive-Copper Nanoparticles

After the synthesis, it is essential to characterize the nanoparticles to determine their size, shape, stability, and other properties. Techniques such as Scanning Electron Microscope analysis (SEM), X-ray diffraction (XRD), dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR) and UV-Vis were used for characterization,

DNA Extraction Method

1- The weight of 50 mg of fresh leaves.

2- The leaves were crushed and transferred to 1.5 ml Ependorf tubes containing 600 microliters of buffer solution (CTAB).
3- The tubes are placed in a water bath at 65 degrees Celsius for 30 minutes.
4- Add 3 microliters of RNase and incubate at 37°C for 60 minutes.
5- Add 400 microliters of isoamylalcohol: chloroform solution at a ratio of 1:24.
6- Mix well and centrifuge at 12000 r/min for 15 minutes.
7- The upper layer is withdrawn and transferred to a new sterile tube, then 600 microliters of isopropanol is added, then centrifuged at 12000 rpm for 10 minutes.
8- The supernatant layer is removed and the precipitated DNA is washed with 600 microliters of 75% alcohol and centrifuged at 7500 rpm for 5 minutes.
9- The supernatant layer is discarded and the DNA is left on air to dry for 30 minutes and 50 microliters of TE buffer is dissolved.
10- DNA is measured using the Nano-Drop device

Statistical Analysis

The data was analyzed successfully using SSPS and ANOVA programsat a significant level 5%.

RESULTS AND DISCUSSION

Characterization of Cuo Nanoparticles

The FTIR spectrum of copper oxide nanoparticles showed bands at 3321 and 1639 cm^{-1}, which are attributed to the stretching vibration of the OH group in water and the bending vibration of H_2O, respectively. The presence of these bands is a preliminary evidence of the formation of CuO nanoparticles, as these materials have a high surface area and therefore have the ability to absorb water on their surfaces [14].

The spectrum also showed bands at 574 and 594 cm^{-1} which are attributed to the stretching vibration of the Cu-O which is the characteristic band for this nano-oxide [15], as shown in Figure 1.

As for figure 2, the UV-Vis spectrum of CuONPs, the peak appear at 220nmwhich confirm the formation of copper nanoparticles and confirm the coordination between copper ion and the organic compounds of olive leaves extract, this result agrees with [16]

In order to determine the nano-size and shape of copper oxide nanoparticles, microscopic measurement was carried out using SEM, and the results proved the presence of irregular geometric structures, which tend to be spherical and sizes ranged from 30-42 nanometers, which indicates that the particles are within the nanoscale size allowed in nanoscience, as presented in Figure 3. The measurement also shows that the particles were not within the quantum size and therefore their use in biological applications is safer.Figure 3 (SEM) shows the homogeneity of the CuONPs which indicates that the synthesis process was carried out in optimum condition and with best yield and quality, which was confirmed by XRD spectrum (Figure 4) and table 1 that showed the particle size was within the range of 4.41-24.74nm with an average of 11.60nm, the results is agreed with [17].

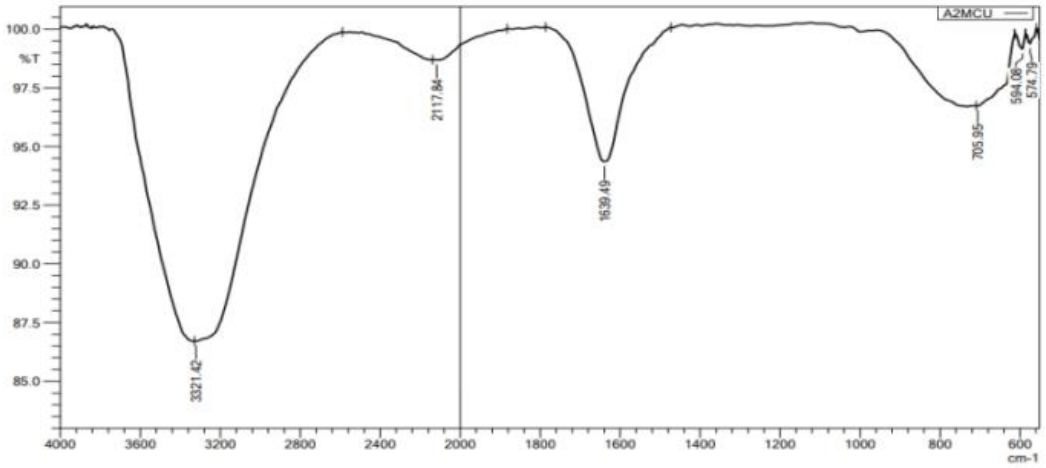

Figure 1: FTIR of CuONPs

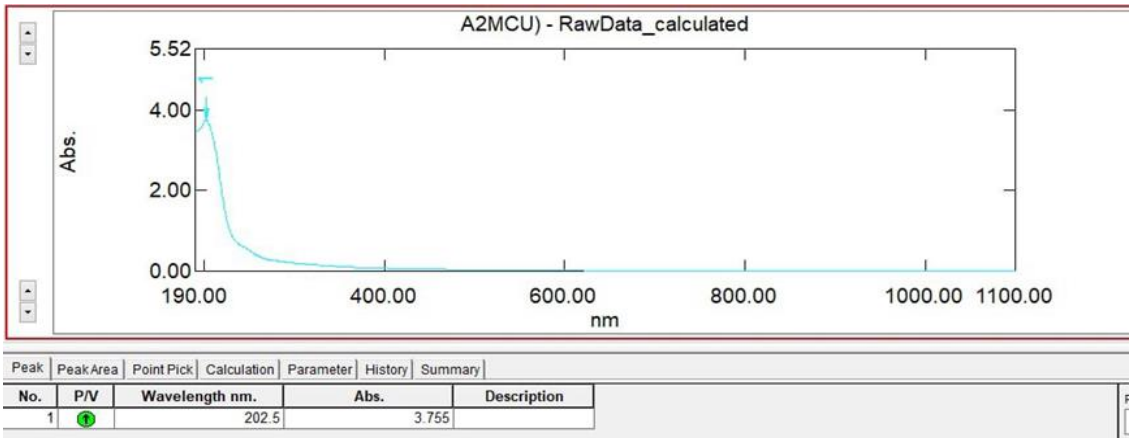

Figure 2: UV-Vis of CuONPs

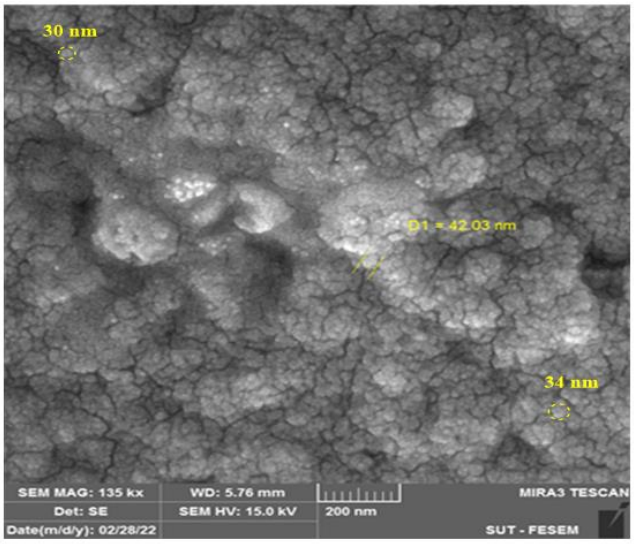

Figure 3: SEM of CuONPs

XRD of Cuonps

The main peaks of copper oxide nanoparticles were recorded by X-ray diffraction. The peaks were situated at 32.7, 35.5, 38.95, 48.65, 53.05, 57.95, 63.5, 66.55 and 74.45degree. These peaks could be attributed to characteristic planes of cubic CuO nanoparticles as shown in Table 1 and Figure 1. Moreover, the particle size of CuO nanoparticles was calculated using Scherrer equation and the results showed that the particle size was within the range of 4.41-24.74nm with an average of 11.60nm as shown in Figure 4 and Table 1.

Table 1: XRD data of CuO nanoparticles

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	h k l	D(nm)	$D_{average}$ (nm)
32.7	6078.8	0.4508	10	19.20	11.60
35.5	4078.2	0.35241	002	24.74	
38.95	1807.4	0.63649	111	13.84	
48.65	1600.2	1.47118	202	6.19	
53.05	2230.2	0.82348	020	11.27	
57.95	1596	1.63265	202	5.81	
63.5	2578.8	0.73212	$\bar{1}13$	13.34	
66.55	1514.8	2.25000	113	4.41	
74.45	1540	1.85000	311	5.64	

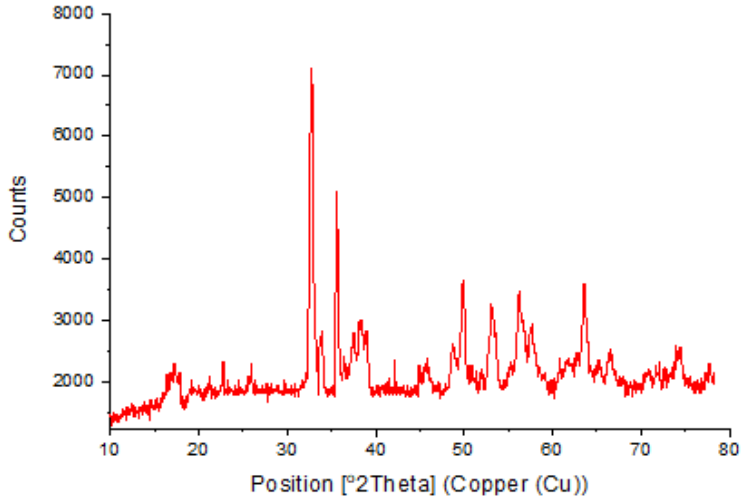

Figure 4: XRD of CuONPs

Phenotypic traits of strawberry seedlings

Phenotypic traits refer to the observable physical characteristics of an organism, in this case, strawberry seedlings, however, some common phenotypic traits of strawberry seedlings includes; leaf shape, leaf color (which at some points may have darker or lighter shades of green), leaf texture (which for strawberry are typically smooth and have a slightly shiny or waxy appearance), root system (which for strawberry seedlings is typically fibrous and shallow, spreading horizontally rather than growing deep into the soil), also the plant height ofstrawberry seedlings are relatively small and compact, with an average height of a few inches to a foot [18].

It's important to note that phenotypic traits can vary among different strawberry varieties, and environmental factors can also influence the expression of these traits [19]. Table 2 shows the effect of CuONPs extract on some strawberry seedlings to its traits. It is clear from table 2Using30% (C3) give best seedling leaf length, and number of fruitswhile number of leaves are best in 20% (C2)compared to other concentrations. While the highest value in the number of flowers in treatmentof bio fertilizer. Best concentration of DNA appears in C3 (134.7) while best DNA purity is found on C2 (20%), however, Statistical analysis results did not shows any significant difference between the treatments and control.

Table 2: The effect of nanoscale copper oxide extract on some phenotypic traits of strawberry seedlings

Treatment	Indicator	Seedling Length	No. of fruits	No. of Leaves	No. of Flowers
C1	Mean ± SD	13.00±8.49	4.50±2.12	19.50±10.61	1.00±1.41
C2	Mean ± SD	15.00±7.07	3.00±1.41	22.50±10.61	1.50±2.12
C3	Mean ± SD	18.75±6.72	8.00±1.41	18.50±9.19	0.50±0.71
F	Mean ± SD	14.25±1.06	4.50±0.71	16.00±7.07	4.50±0.71
Control	Mean ± SD	19.25±6.72	5.50±2.12	19.00±1.41	3.50±0.71
LSD	Sig.	0.825 NS	0.167 NS	0.955 NS	0.092 NS
C1: 10% ; C2 :20% ; C3:30% ; F: vital fertilizer; NS: Not Significant					

Effect of Olive Leaves Extract on the Genotype of Strawberry

The effect of olive leaves extract on the genotype of strawberries is not well-documented and would likely depend on various factors such as the concentration and application method of the extract, the specific genotype of the strawberry plant, and the duration of exposure.While olive leaves extract contains various bioactive compounds, such as phenolic compounds, flavonoids, and antioxidants, which may have potential effects on plant growth and development, including modulation of gene expression, the specific impact on the genotype of strawberries would require further scientific investigation [20].

It's worth mentioning that genotypic traits are primarily determined by the plant's genetic makeup, and while environmental factors and certain treatments can influence gene expression, they generally do not directly alter the genotype. Changes in gene expression can occur due to environmental stressors or the application of certain compounds, Table 3 shows that best concentration of DNA appears in C3 (134.7) while best DNA purity is found on C2 (20%) as depicted by (Hermans *et al.*, 2020) [21].

Table 3: Effect of the extract on the genotype of strawberry

No.	Genotype	DNA Concentration	DNA Purity
1	C1	115.5	1.90
2	C2	120.6	1.82
3	C3	134.7	1.74
4	CONT.	34.1	1.16
5	F1	582.6	1.78
Con : control ; C1: 10% ; C2 :20% ; C3:30% ; F: vital fertilizer			

The Effect of Olive Extract on the Quantity of Chlorophyll in Leaves of Strawberry

The effect of olive extract on the quantity of chlorophyll in strawberry leaves has not been extensively studied, and there is limited scientific literature available on this specific topic. However, I can provide you with some general information regarding the potential effects of plant extracts on chlorophyll content in leaves [22].

Chlorophyll is a pigment essential for photosynthesis, which enables plants to convert light energy into chemical energy. Changes in chlorophyll content can be indicative of alterations in photosynthetic activity and plant health [23].While olive leaf extract contains bioactive compounds that may have various effects on plants, including potential impacts on chlorophyll content, the specific outcome would depend on factors such as the concentration and application method of the extract, the strawberry variety, and environmental conditions [24].

Table 4: shows the effect of the extract on the quantity of chlorophyll in leaves of strawberry seedlings, the chlorophyll was measured using SPAD (Soil Plant Analysis Development) which shows optimum with C3 (62.63) this result agreed with [25]. The statistical analysis shows a significant correlation p-value ≥ 0.05, for C1, C3, and F (values were 0.044, 0. 0.000, and 0.006) compared with control.

Table 4: Effect of the extract on the amount of chlorophyll in the leaves of strawberry seedlings

Treatment	**Mean±SD**	**LSD**
C1	38.9±2.62	0.044*
C2	48.4±5.47	0.306NS
C3	62.63±3.5	0.000**
F1	55.13±2.73	0.006**
Control	45.37±1.64	---
The chlorophyll was measured using SPAD *Con : control ; C1: 10% ; C2 :20% ; C3:30% ; F: vital fertilizer*		

CONCLUSIONS

1. CuO nanoparticles were synthesized successfully.
2. Different concentrations were prepared from olive leaves abstract.
3. Novel green CuO nanoparticles were prepared from olive extract and CuO nanoparticles.
4. The novel green synthesized NP was applied in different concentration on the germination of strawberry.
5. Best concentration that gives best seeding and best chlorophyll is 30% (C3).
6. Significant correlation between extract and chlorophyll at C1, C3, and F

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