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The Effect of Some Nanoparticles Fertilizers on the Chemical Characteristics of Growing *Ruta graveolens* L. Plant in *invivo*

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ABSTRACT

The studies included tow experiments, the laboratory and the field experiment, for the period from 7/10/2018 to 1/4/2019, to study the effect of some nanoparticles fertilizers in growth of *Ruta graveolens* L. *invivo*, The laboratory experiment was performed in the laboratories of College of Education for Pure Science / University of Diyala, nanoparticles identified by chemical and physical methods, included measurement of X-Ray Diffraction (XRD), Energy Dispersive X- ray Spectroscopy (EDX) and Scaning Electron Microscope (SEM). The results showed that the average granular size of ZnO and CuO was 32.52 and 23.48 nm respectively. The field experiment carried out in the field belong to house garden. The experiments was designed using a completely randomized design (CRD), each treatment replicated five times for field experiment and five samples for each treatment, this experiment included seven different treatment, control treatment (without Zno and CuO nanoparticles), 25 mg/l ZnO, 50 mg/l ZnO, 25 mg/l CuO, 50 mg/l CuO, 12.5+12.5 mg/l ZnO+ CuO, 25+25 mg/l ZnO+ CuO. The comparison between the mean of the treatment was done using Duncan at 0.05 level of significance. the results showed that zinc oxide and copper oxide granular size of 32.52 and 23.48 nm, as it was diagnosed using the technique of diffraction X-Ray and the use of the Debye – Scherrer equation. as well as that the prepared material of ZnO and CuO is pure material has been diagnosed using the Energy Dispersive X-ray Spectroscopy (EDX). Use of ZnO + CuO 25 + 25 $mg.l^{-1}$ concentration caused significantly influenced in the leaves content of chlorophyll and at a rate of 2.96 SPAD unit compared with the control treatment which gave a rate of 1.77 SPAD unit. and the same treatment caused an increase in the percentage of protein at rate of 13.62% and at rate of 17.87% for carbohydrates While the control treatment gave a percentage of 12.40% for protein and 13.18% for carbohydrates. While the treatment 12.5+12.5 mg/l ZnO+ CuO showed an increased in the leaves content of Auxin at a rate of 9.93 mg/kg compared with the control treatment which gave a rate of 8.40 mg/kg.

KEYWORDS

ZnO, CuO Nanoparticles, *Ruta graveolens* L, *invivo*, X-ray, EDX.

Introduction

Ruta graveolens L. is a perennial plant that belongs to the family Rutaceae. The Mediterranean region is the original home of the plant, and from it its cultivation spread to many countries of the world (Al-Kateb, 2000). The plant has medicinal properties that it has been used since ancient times by the Greeks and Romans (Pollio et al., 2008) as a medicine for treating various conditions such as eye problems, rheumatism, dermatitis, pain and many inflammatory conditions (Ratheesh and Helen, 2007). The plant contains many secondary metabolites that are used in the pharmaceutical industries, including acridone alkaloids, coumarins, volatile substances, terpenoids, flavonoids, furoquinoline, saponins, tanins, and glycosides (Hashemi et al., 2011). An important development in foliar spraying is the use of nanotechnologies, as nanofertilizers, when used, have the advantage of avoiding toxic effects in plants through a slow and more specialized delivery of micronutrients, while reducing potential soil pollution and other environmental risks that may occur when using chemical fertilizers added directly to the soil. (Solanki et al., 2015). In addition, it is added in smaller quantities than conventional fertilizers (Davarpanah et al., 2016). Zn is an essential micronutrient that plants need in small amounts. It is important for plant growth and development because it plays an important role in a wide range of life processes (Adiko et al., 2017). This micronutrient is essential for a wide range of physiological and biochemical processes such as photosynthesis, protein synthesis, antioxidant, pollination, growth regulation, and in the synthesis of tryptophan which is the basis of IAA and which has an active role in the production of the hormone Auxin essential for growth (Hafeez et al., 2013 and Narendhran et al., 2016). ZnO NPs, in particular, are environmentally friendly, provide easy action, non-toxic, biocompatible and biocompatible making them ideal candidates for biological applications (Mohammad et al., 2010). In plants, copper plays a major role in chlorophyll formation, photosynthesis and the electron transport chain in the mitochondria and protection against

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oxidative stress as well as protein, carbohydrate and cell wall metabolism. Therefore, copper deficiency can alter various functions in plant metabolism (Rehman et al., 2019). This study aims to Study of the effect of spraying with zinc oxide and copper oxide nanoparticles on the chemical growth characteristics of *Ruta graveolens* L. plants.

Materials and Methods

Preparation of Zinc Oxide

Zinc oxide nanostructures were prepared by the Co-Precipitation method reported by Ahamed and Kumar (2016). 0.1 M aqueous zinc acetate was prepared and the solution was placed on a hot plate magnetic rotor at room temperature and drops of NaOH were added to it at a concentration of 0.1 Mm. Drops until the pH is 12, after which the temperature of the solution was raised to 80 ° C for the purpose of evaporation of the excess water. The remaining solution was filtered with a filter paper and the sediment was washed twice with ethanol and three times with distilled water. The sediment was dried by an electric oven for two hours at a temperature of 80 ° C and burned At a temperature of 450 ° C for two hours.

Preparation of Copper Oxide

2.9 g of aqueous copper nitrate Cu (NO3) 2.3H2O was dissolved in 100 ml distilled water and put the solution on a magnetic stirrer for 30 minutes until the material completely dissolved. 1.2 g of Polyvinylpyrrolidone (PVP) was added to it, then stirred and heated until reaching The temperature is 60 ° C. After reaching this temperature, sodium hydroxide was added at a 1 M concentration by droplet until the pH reached 9. Then the solution was stirred for one hour until a black precipitate was formed. The precipitate was filtered and washed using distilled water and ethanol alcohol. Then the sediment was dried for 30 minutes at a temperature of 80 ° C. Then the sediment was burned at 500 ° C for 3 hours (Mayekar et al., 2014).

Characterization Techniques Nanoparticles

The metal oxides nanoparticles were depicted by a couple of systems including X-pillar diffraction (XRD), Energy Dispersive X-beam Spectroscopy (EDX) and Scanning electron amplifying instrument (SEM). Crystallinity, structure, and crystallite size of nanoparticles were constrained by XRD using a Shimadzu (Kyoto, Japan) Miniflex X-pillar diffractometer with Cu-Kα radiations (λ = 0.15406 nm) in the 2θ region from 20° to 80°. (EDX) Energy Dispersive X-beam Spectroscopy at the University of Kashan/Iran. SEM evaluation was finished using a 200 kV Zeiss (Germany) checking electron amplifying the focal point.

Field Experiment

The field experiment was conducted in the home garden, as the plants obtained from the agricultural nursery of the Diyala Agriculture Directorate were rotated into plastic pots with a diameter of 25 cm on (01/10/2018) containing peat moss, sandy soil and mix soil at a ratio of 1: 2: 1. Spraying plants in the vegetative growth stage with ZnO and CuO at 3 concentrations 0, 25 and 50 mg. L^{-1} and a combination thereof at a concentration of 12.5 mg. L^{-1} of zinc oxide and copper oxide nanoparticles and a concentration of 25 mg. L^{-1} of oxide Zinc and copper oxide nanoparticles that were prepared in a laboratory as mentioned previously, with two sprays, the first spray on 1/12/2018 and the second spray 30 days after the first spray. Watering and weeding were carried out when needed. The plants were sprayed with an insecticide Top Ride containing the organic substance Acetamipride with a concentration of 25%, as 1 gm of the substance was dissolved in 1 liter of water.

Chemical Characteristics Studied of *Ruta Graveolens* L. Plant

The measurement Chemical characteristics of ***Ruta graveolens* L.** plant were as shown in Table (1).

Table 1. Methods of measured Chemical characteristics of *Ruta graveolens* L.plant

No	Chemical characteristics	Method of measurement
1	Chlorophyll (SPAD Unit)	measured by method (Loh *et al*, 2000)

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2	Protein %	measured by method (Thachuk *et al*, 1977)
3	Carbohydrates %	measured by method (Pearson *et al*, 1976)
4	Auxin Mg/ kg	measured by method (Nuray, *et al* 2002)

Results and Discussion

Characteristics of (ZnO) and (CuO) Nanoparticles

X-Ray Diffraction of ZnO Nanoparticles

Figure (1) shows the X-ray diffraction patterns for the nanosynthesis of zinc oxide, as the results in this figure showed that the highest peaks of diffraction angles are (31.78, 34.42 and 36.26) 2θ Table (2) and that these peaks indicate the nature of the crystal structure of ZnO. These peaks matched the standard value base for nanostructured zinc oxide (JCDPS ZnO) and these results are in agreement with Adeela et al (2015).

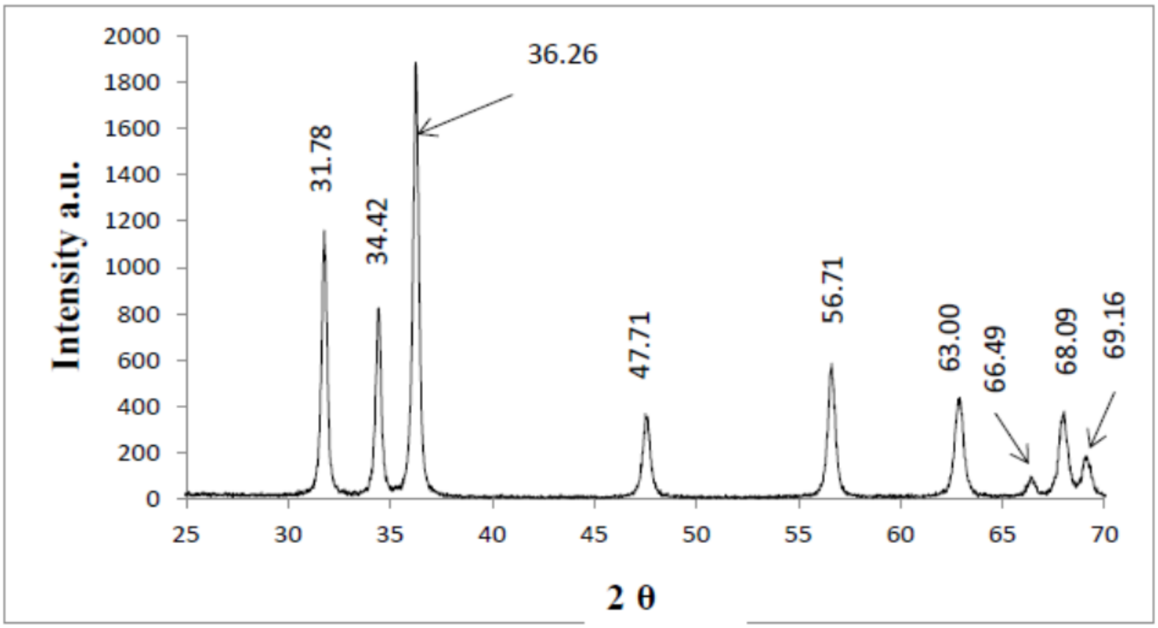

Figure 1. XRD patterns of zinc oxide nanoparticles

Table 2. The values of X-ray diffraction and average grain size of nanostructured Zinc Oxide

No	Peak position 2θ (°)	FWHM B size (°)	Dp (nm)	Dp Average (nm)
1	**36.2650**	**0.2706**	**31.058**	**32.52**
2	**31.7862**	**0.2713**	**30.648**	
3	**34.4295**	**0.2328**	**35.868**	

X-Ray Diffraction of CuO Nanoparticles

Figure (2) shows the X-ray diffraction patterns for the composition of the copper oxide nanoparticles, as the results in this figure showed that the highest peaks of the diffraction angles are (35.55, 38.77 and 48.77) 2θ Table (3) and that these peaks indicate the nature of the crystal structure of CuO as they match These peaks correspond to standard base

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values for nanostructured zinc oxide (JCDPS ZnO) and these results are in agreement with Adeela et al (2015).

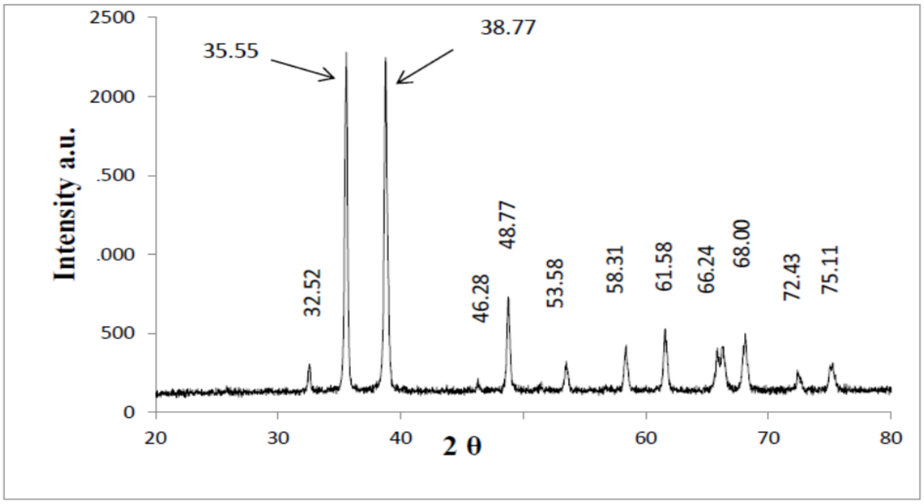

Figure 2. XRD patterns of copper oxide nanoparticles

Table 3. The values of X-ray diffraction and average grain size of copper oxide nanoparticles

No	Peak position 2θ (°)	FWHM B size (°)	Dp (nm)	Dp Average (nm)
1	38.7763	0.4206	20.101	23.48
2	35.5525	0.3223	25.988	
3	48.7706	0.3593	24.376	

Energy Dispersive X- ray Spectroscopy analysis (EDX)

EDX of ZnO Nanoparticles

The energy dispersive X-ray spectrum EDX was used to determine the formation of the nano zinc oxide ZnO, as shown in Figure (3), as the energy dispersive X-ray spectrum in the product contains only the elements Zn and oxygen O, and no other elements in the spectrum were detected indicating the degree High purity zinc oxide nanoparticles.

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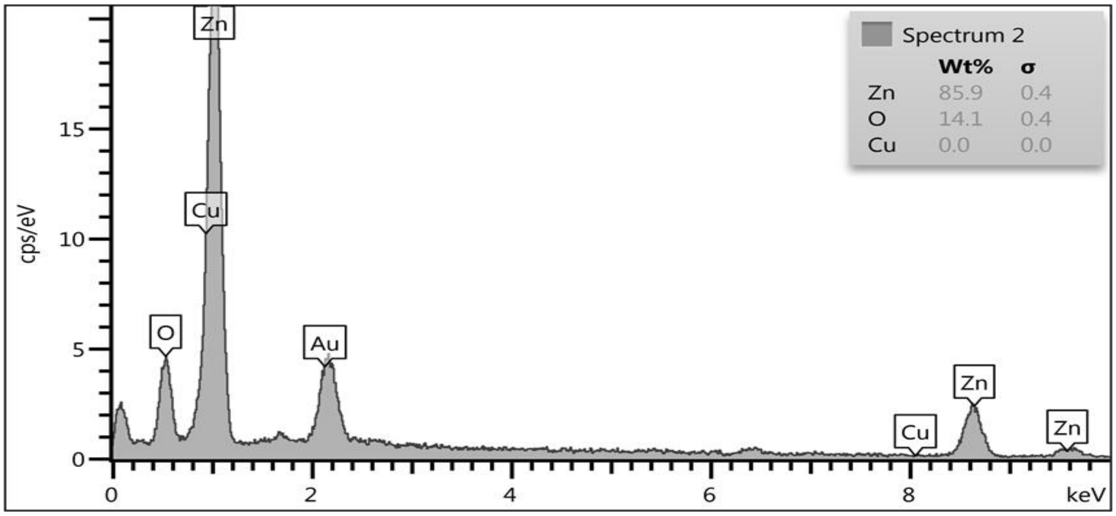

Figure 3. EDX curve for nanostructured zinc oxide

EDX of CuO Nanoparticles

The energy dispersive X-ray spectrum EDX was used to determine the formation of the nano copper oxide CuO, as shown in Figure (4), as the energy dispersive X-ray spectrum in the product contains Cu and O and a very small percentage of Zn, which indicates the degree of purity of the particles. Premium Copper Oxide Nanoparticles.

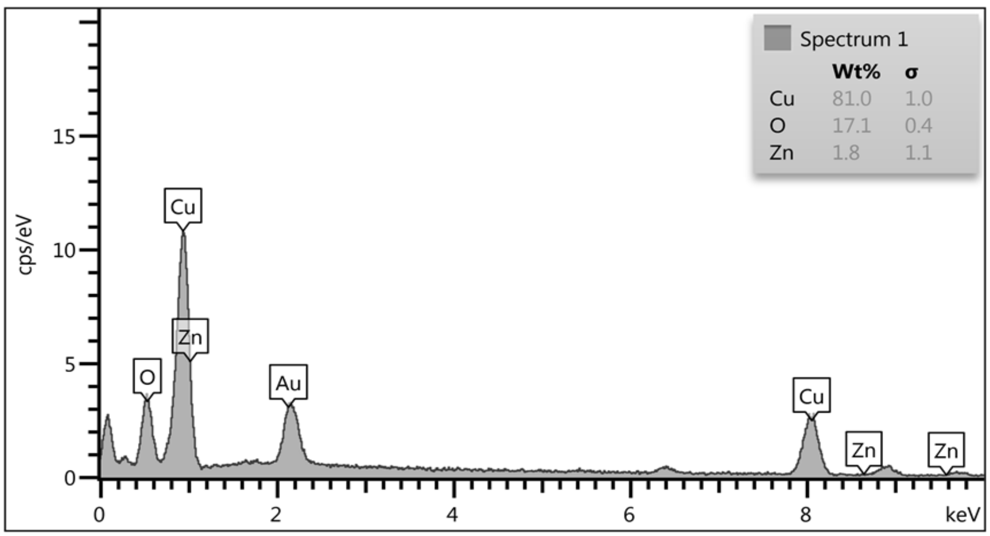

Figure 4. EDX curve for copper oxide nanoparticles

Scanning Electron Microscopy Analysis (SEM)

SEM of ZnO Nanoparticles

Figure (5) show the scanning electron microscopy images of the ZnO nanoparticles sample.

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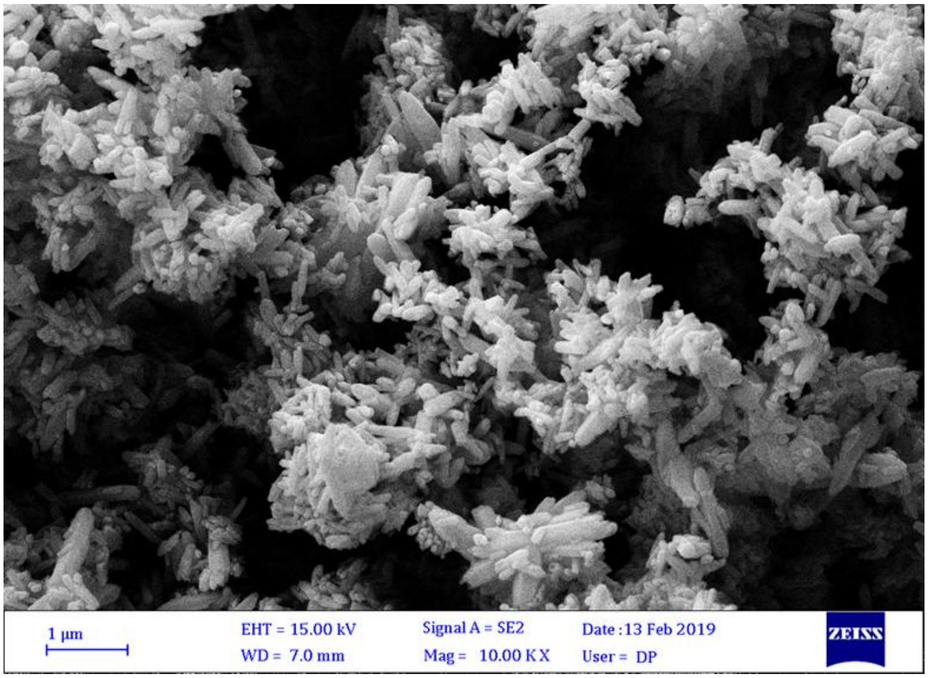

Figure 5. Scanning electron microscope images of Zinc Oxide nanoparticles

SEM of CuO Nanoparticles

Figure (6) show the scanning electron microscopy images of the CuO nanoparticles sample.

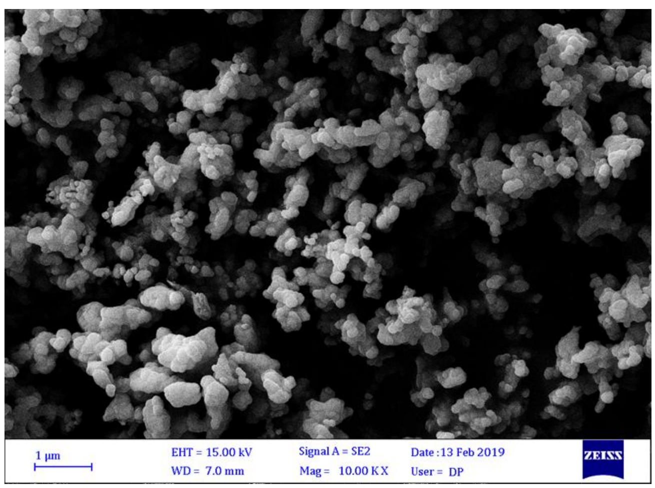

Figure 6. Scanning electron microscope images of copper oxide nanoparticles

Chemical Characteristics of *Ruta Graveolens* L. Plant

The results showed In Table (3) that the leaves content of chlorophyll was at a rate of 2.96 SPAD unit compared with the control treatment which gave a rate of 1.77 SPAD unit. And this caused an increased in nutrient concentrations in leaves at rate of 13.62% of protein,17.87% for carbohydrates While the treatment of control gave a percentage of 12.40% and 13.14% respectively. While the treatment 12.5+12.5 mg/l ZnO+ CuO showed an increased in the leaves content of Auxin at a rate of 9.93 mg/kg compared with the control treatment which gave a rate of 8.40 mg/kg. Nanomaterials play roles in improving the efficiency of nutrient uptake (Tripathi et al, 2018). The reason for the significant increase in the evidence of chlorophyll in papers when adding nano zinc oxide is due to the role of zinc in the biological reactions responsible for the formation of chlorophyll, as zinc participates in building and manufacturing chlorophyll indirectly through its direct role in the production of energy compounds and building amino acids (Ruttkay-Nedecky et al., 2017). Zinc increases the ability of plants to absorb phosphorous and acts as a cofactor for the enzyme carbonic anhydrase that regulates the pH inside the green plastid as it enables proteins to maintain their biological nature, as well as the role of zinc as a cofactor and antioxidant (Jacqueline et al., 2012). Copper contributes to plant growth and is also involved in building green plastids (Wang et al., 2004). The reason for the significant increase.

Table 4. The effect of adding different concentrations of zinc oxide and copper oxide nanoparticles to the chlorophyll index, carbohydrate percentage, protein percentage and auxin content in *Ruta graveolens* L. grown in the field

treatments	Chlorophyll (SPAD Unit)	Carbohydrates %	Protein %	Auxin Mg/kg
0+0 mg.l^{-1}	1.77 e	13.18 d	12.40 f	8.40 e
ZnO 25 mg.l^{-1}	2.42 c	16.28 bc	13.12 cd	9.40 c
ZnO 50 mg.l^{-1}	2.49 c	16.90 abc	13.18 bc	9.50 bc
CuO 25 mg.l^{-1}	2.28 d	15.58 c	13.02 de	9.36 c
CuO 50 mg.l^{-1}	2.22 d	15.52 c	12.99 e	9.03 d
ZnO+CuO 12.5+12.5 mg.l^{-1}	2.82 b	17.29 ab	13.26 b	9.93 a
ZnO+CuO 25+25 mg.l^{-1}	2.96 a	17.87 a	13.62 a	9.66 b

*** Values with similar characters do not different significantly from each other.**

the percentage of carbohydrates and protein in the leaves is due to the role of nanocomposites in increasing the plant's ability to absorb the elements, the most important of which is nitrogen (Thakur et al., 2018). The increase in the leaves ’nitrogen content led to an increase in their protein content, as zinc participates in the nitrogen metabolism process inside The plant stimulates the enzyme RNA polymerase that enhances the construction of RNA and protein (Al-Qazzaz, 2014). Zinc contributes to the synthesis and production of proteins through the chemical catalysis process and helps maintain protein structure and stability (Kisan et al., 2015). The copper component has an important role in the protein synthesis process as it increases the plant’s ability to fix atmospheric nitrogen, as well as stimulate the plant to form nucleic acids and protein (Al-Naimi, 1999). The reason for the significant increase in auxin is attributed to the important role of zinc in building and manufacturing the amino acid tryptophan, which is the main substance for auxin production in plants (Barlog et al., 2016).

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