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Morphological and Biochemical responses of Coffee Seedlings (*Coffea arabica* L.) to Foliar Application of ZnO and ZnO/Ag Nanoparticles
Respuestas Morfológicas y Bioquímicas de Plántulas de Café (*Coffea arabica* L.) a la Aplicación Foliar de Nanopartículas de ZnO y ZnO/Ag

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SUMMARY

The application of nanotechnologies in agriculture has gained in importance in recent years, and their impact on plant growth and development has been documented with successes and limitations. In the case of coffee cultivation, it is a viable alternative compatible with mineral fertilization schemes. The objective of this research was to study the physiological effect of zinc oxide nanoparticles (ZnONPs) and silver-impregnated zinc oxide (ZnONPs/AgNPs), synthesized through the sol-gel method on vegetative growth, total chlorophyll concentration, and the synthesis of functional groups in coffee (*Coffea arabica* L.) seedlings. The nanoparticles were characterized by X-ray diffraction (XRD) and applied to the leaves. Plant height, stem diameter, leaves per plant, and leaf area, concentration of total chlorophylls and functional groups were measured by FT-IR spectroscopy. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the synthesized nanoparticles, which showed sharp peaks consistent with the hexagonal wurtzite structure, while the ZnONPs/AgNPs showed structures corresponding to the cubic phase of metallic silver. A 58.18% increase in plant height was observed upon application of ZnONPs compared to the control, and 34.78% increase in the number of leaves per plant. The highest chlorophyll concentration was recorded when combining ZnONPs+2.5AgNPs plus fertilizer, with an increase of 81.8% with respect to the control. The combined treatment of ZnONPs/AgNPs with fertilizer significantly increased the accumulation of compounds such as caffeine, sucrose, and carbohydrates. These results demonstrate the potential of nanofertilizers as growth promoters and modulators of functional metabolism in coffee seedlings, although direct parameters of productivity and bean quality were not evaluated.

Index words: *FT-IR, growth promoter, nanofertilizers, ZnO nanoparticles.*

RESUMEN

La aplicación de nanotecnologías en la agricultura ha cobrado auge en los últimos años y su impacto en el crecimiento y desarrollo de las plantas se ha documentado con aciertos y limitaciones. En el caso del cultivo de café es una alternativa viable compatible con los esquemas de fertilización mineral. El objetivo de esta investigación fue estudiar el efecto fisiológico de nanopartículas de óxido de zinc (ZnONPs) y óxido de zinc impregnado con plata (ZnONPs/AgNPs), sintetizadas a

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través del método de sol-gel en el crecimiento vegetativo, la concentración total de clorofilas y la síntesis de grupos funcionales en plántulas de café (*Coffea arabica* L.). Las nanopartículas se caracterizaron por difracción de rayos X (DRX) y se aplicaron a las hojas. Se midieron la altura de la planta, diámetro del tallo, hojas por planta y área foliar, concentración de clorofilas totales y grupos funcionales a través de espectroscopía FT-IR. El análisis de difracción de rayos X (DRX) confirmó la naturaleza cristalina de las nanopartículas sintetizadas, las cuales mostraron picos agudos consistentes con la estructura hexagonal wurtzita, mientras que las ZnONPs/AgNPs mostraron estructuras correspondientes a la fase cúbica de la plata metálica. Se observó un incremento del 58.18% en la altura de la planta al aplicar ZnONPs en comparación con el control y de 34.78% en el número de hojas por planta. La mayor concentración de clorofilas se registró al combinar ZnO+2.5Ag más fertilizantes con incremento de 81.8% respecto al control. El tratamiento combinado de ZnONPs/AgNPs con fertilizante aumentó significativamente la acumulación de compuestos como cafeína, sacarosa y carbohidratos. Estos resultados demuestran el potencial de los nanofertilizantes como promotores del crecimiento y moduladores del metabolismo funcional en plántulas de café, aunque no se evaluaron parámetros directos de productividad y calidad del grano.

Palabras clave: *FT-IR, promotor de crecimiento, nanopartículas de ZnO, nanofertilizantes*.

INTRODUCTION

Obtaining quality coffee seedlings that guarantee their survival during and after transplanting is very important to ensure greater development of the plant's root and aerial organs (Campos *et al*., 2019). This crop is the second most traded commodity in the world (DaMatta, Ronchi, Sales, and Araújo, 2007; Alonso-Salces, Serra, Reniero, and HÉberger, 2009; Hong *et al*., 2024; Ruse *et al*., 2025), not only for its commercial value but also for what it represents as a livelihood for thousands of families internationally and particularly in the Frailesca region of Chiapas, Mexico (Flores-Vichi, 2015; Venegas-Sandoval, Soto, Herrera, and Álvarez, 2020; Fonseca-Castillo, Campos, Prado, Rodríguez, and La O, 2025).

By the year 2023, world coffee production reached approximately 168.2 million 60 kg bags, representing a decrease of 0.1% compared to coffee year 2022 and 1.5% less compared to 2020/2021 (ICO, 2024). This indicates that coffee cultivation depends on a series of factors, including climatic factors, latitude, soil types, shade, and management practices, among which fertilization plays a crucial role in guaranteeing increases in yields and fruit quality (Meléndez-Mori, Lapiz, Huaman, Zuta, and Oliva, 2025).

In this regard, there is currently a growing interest in sustainable technologies that improve plant productivity while minimizing ecological impact. Among these, nanotechnology has emerged as an innovative and novel alternative for contemporary agriculture, as it ensures greater efficiency in nutrient uptake, increased plant resistance to pests and diseases, and reduced dependence on chemical inputs (Gupta, Rayeen, Mishra, Tripathi, and Pathak, 2023; Yousef *et al*., 2023; Nguyen *et al*., 2024; Tang *et al*., 2024).

The application of silver nanoparticles (AgNPs) has attracted increasing attention due to their multiple benefits for plant growth. In addition to their demonstrated effects on plant germination, growth, and development (Ma, Geiser, Deng, and Kolmakov, 2010a; Spinoso-Castillo *et al*., 2017; Hu and Xianyu, 2021; Tung *et al*., 2021), they possess antimicrobial properties, which reduce the incidence of pathogenic diseases (Pokhrel *et al*., 2012). Likewise, AgNPs improve plant tolerance to adverse conditions such as salinity, drought, or use of low quality irrigation water, and can be further enhanced when combined with beneficial soil microorganisms (Alfosea-Simón, Burgos, and Alburquerque, 2025).

Zinc and ZnONPs not only increase crop growth and yield, but also improve resistance under adverse conditions, making them a key tool for more sustainable and efficient agriculture (Wang *et al*., 2023). Reddy (2021) reports that the critical levels of Zn in soil and plant range from 15 mg kg^{-1} and 10-20 mg kg^{-1} (plant dry mass), respectively. In this sense, Rivera-Gutiérrez *et al*. (2021) demonstrated that doses of 200 mg L^{-1} of ZnONPs increased yield in melon (*Cucumis melo* L.) plants with direct effects on nutraceutical content and fruit Zn concentration. Estrada-Arellano *et al*. (2023) concluded that the foliar application of ZnO nanoparticles can effectively reduce Zn deficiency in pecan tree (Carya illinoinensis), thereby significantly increasing yield; however, the nutraceutical quality is affected, since the influence of these nanoparticles significantly increases saturated fatty acids and decreases unsaturated fatty acids, in addition to the percentage of crude protein.

However, it has been found that the application of ZnONPs can have both positive and negative effects on plant physiology. For example, Meléndez-Mori *et al*. (2025) found that the application of ZnO-NPs to salt-stressed coffee plants had both positive and negative effects. An increase in proline content from 33% to 77% was detected in stressed plants treated with ZnO-NPs, in contrast to unstressed plants without application. Catalase enzyme activity increased with ZnO-NPs application compared to plants subjected to salt stress without treatment. On the other hand, the application of ZnONPs decreased H_2O_2 levels by up to 18.7% with respect to the control group. On the other hand, a 45% higher Na^+ accumulation was observed in NaCl-stressed seedlings treated with ZnONPs (Meléndez-Mori *et al*., 2025). This is why given the complex interaction between ZnONPs application and several physiological processes in coffee plants, including photosynthesis, a detailed analysis is required to fully understand the response of coffee plants to ZnONPs application (Meléndez-Mori *et al*., 2025).

The use of silver in the form of nanoparticles, either alone or impregnated with other nanoparticles, as is the case of Zn, has been documented in several studies. Bello-Bello and Spinoso (2023), in a literature review on the application of AgNPs in plant micropropagation, draw attention to the hormetic effect this causes in plants, which should be taken into account when defining application doses to avoid low or excessive doses that affect plant growth and development.

Therefore, the objective of this research was to study the effect of zinc oxide nanoparticles impregnated with silver and combined with mineral fertilization on growth, total chlorophyll concentration, and synthesis of functional groups in coffee seedlings.

MATERIALS AND METHODS

Synthesis of ZnONPs/AgNPs

The synthesis of ZnONPs was carried out by the sol-gel method, for which a 0.29 M solution of zinc nitrate hexahydrate ($Zn(NO_3)_2 \bullet 6H_2O$ Fermont, Monterrey, Mexico) diluted in methanol CH_3OH (Meyer, Mexico City, Mexico) was prepared. This solution was stirred at 350 rpm at room temperature until pH=4 was reached. A 0.5 M NaOH solution (Fermont, Monterrey, Mexico) was prepared in distilled water with an ultrasonic bath to facilitate dissolution. Subsequently, the NaOH solution was added dropwise to the zinc nitrate. When the pH stabilized at 12, it was left to stand for twenty hours. The product obtained was carefully washed to remove excess water. The resulting product was dried at 50 °C in a flask (Thermo Scientfic Waltham, MA, USA) and then calcined at 500 °C for two hours to obtain ZnONPs (Robledo, Enríquez, Avendaño, Hernández, and Gutiérrez, 2023).

Impregnation in Silver

This process was carried out through the incipient wet impregnation method (Pérez-Madrigal *et al*., 2024). For this purpose, $AgNO_3$ (Fermont, Monterrey, Mexico) was weighed at concentrations of 1.5 and 2.5%, respectively, and 3 mL of distilled water was added to each concentration. The obtained mixtures were diluted in an ultrasonic bath (Fisher Scientific, Waltham, MA, USA) and placed in crucibles containing ZnONPs and dried on a hot plate stirrer (Cole Parmer, Vernon Hills, IL, USA) at 100 °C for three hours (Pérez-Madrigal *et al*., 2024).

Characterization of ZnO/Ag NPs

The ZnONPs/AgNPs were characterized by X-ray diffraction (XRD) method. For this purpose, an Ultima IV diffractometer (Rigaku, Tokyo, Japan) operated at 40 kV and 44 mA in Bragg-Brentano mode with a Cu X-ray source (λ Kα = 0.15419 nm) was used. Diffraction patterns were recorded from 20° to 80° with a step size of 0.02° and a scanning speed of 0.2° min^{-1}.

Plant Waterial

Seeds of *Coffea arabica* L. var. Costa Rica 95) were sown in 64-cavity polyethylene trays on a substrate composed of fine sand. Seedlings were transplanted when they reached an average height of 5.3 cm in black polyethylene bags, 400 caliber, 20 × 30 cm, using as substrate mountain soil and sand (60:40 v/v) and pH of 5.8. Irrigation was done daily, and weed control was done manually every 15 days. A basal fertilization with NP (18-46) was carried out at a dose of 4 g per plant, divided into 2 g 40 days after germination and the same interval of days after the first dose.

For foliar application of the nanoparticles, solutions containing ZnONPs, ZnONPs/1.5%AgNPs, and ZnONPs/2.5%AgNPs were prepared at a concentration of 100 mg L^{-1} in deionized water. To ensure homogeneity, an ultrasonic bath (Fisher Scientific, Waltham, MA, USA) was used for 25 min. Foliar application was done manually once a week, applying 1 mL of solution per plant. The volume of 1 mL was measured with a precision micropipette and corresponds to complete coverage of the leaf surface at the seedling stage, without runoff. The average droplet size in the foliar spray was estimated at approximately 100 μm, ensuring a homogeneous distribution on the leaf surface (Parisi, Vigani, and Rodríguez, 2015).

Plant Growth

Plant growth was evaluated based on variables including plant height (cm) measured with a millimeter ruler from root collar to stem apex, stem diameter in mm measured 5 cm from the base using a caliper, and leaf area determined indirectly using the formula proposed by Rodriguez-Larramendi *et al*. (2016).

$$LA = L \bullet W \bullet K \tag{1}$$

Where: LA = Leaf area; L = Leaf length; W = Leaf width; K = Leaf coefficient (0.65).

Total Chlorophyll Concentration

Total chlorophyll content was measured with a portable chlorophyll meter (MC-100, Apogee Instruments, Logan, UT, USA). For the determination, samples were taken from a fully expanded leaf from the middle part of each plant, avoiding the veins. All growth and chlorophyll variables were measured on 10 randomly selected plants per treatment.

Determination and Characterization of Functional Groups

The determination and characterization of functional groups was performed by Fourier transform infrared spectroscopy (FT-IR). An FT-IR spectrophotometer (Thermo Scientific NICOLET, IS50, MA, USA), equipped with a diamond tip ATR accessory, was used. Functional groups were obtained from dried root, stem, and leaf samples in a spectrum range of 4000 to 600 cm^{-1}, resolution of 4 cm^{-1}, and accumulation of 16 scans in transmittance mode (Parmar, Kumar, and Sinngh, 2019).

Experimental Design

A completely randomized design was used with eight treatments and ten replications, totaling 80 experimental units composed of one plant per bag. The treatments consisted of the application of zinc oxide nanoparticles impregnated in variable amounts with silver (Ag) and mineral fertilizer, which were established as follows: ZnONPs + Fertilizer, ZnONPs, ZnONPs + 1.5 AgNPs + Fertilizer, ZnONPs + 1.5 AgNPs, ZnONPs + 2.5 AgNPs + Fertilizer, ZnONPs + 2.5 AgNPs + Fertilizer, and a control treatment.

Statistical Analysis

One-way analysis of variance was performed on the plant growth variables for a probability of error of 5% ($p \leq 0.05$), after comparison of the assumptions of normality and homogeneity of variance. Comparison of means was performed through Tukey's test ($p \leq 0.05$). The analyses were performed with STATISTICA software, version 8.0 (StatSoft, 2008).

RESULTS AND DISCUSSION

X-ray Diffraction (XRD) of ZnONPs and ZnONPs/AgNPs

X-ray diffraction (XRD) analysis confirmed the crystalline nature of the synthesized nanoparticles. ZnONPs exhibited sharp peaks consistent with the hexagonal wurtzite structure (PDF 00-036-1451), while ZnONPs/AgNPs additionally showed peaks at $2\theta = 38.2°$, 44.4°, and 64.6°, corresponding to the cubic phase of metallic silver (PDF 01-087-0720) (Figure 1). These results are consistent with Ma *et al*. (2010b), who also observed distinct crystalline structures for ZnONPs and AgNPs synthesized via sol-gel. Rietveld refinement quantified Ag impregnation levels of 1.4% and 2.2%, showing that silver incorporation reduced particle size, improving dispersion and reactivity (Naseer and Iqbal, 2024). Smaller nanoparticles are favored for foliar uptake due to higher surface area and ease of penetration into leaf cuticles and stomatal pores (Singh, Singh, Afzal, Singh, and Hussain, 2018).

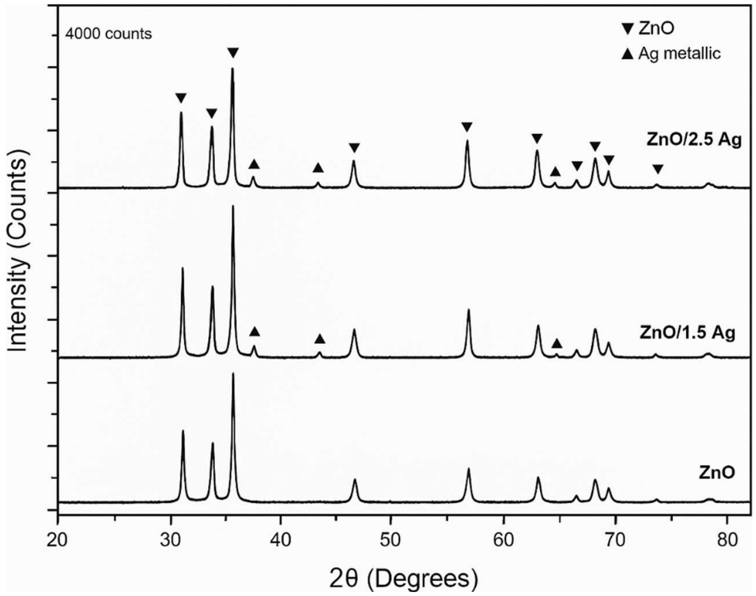

Figure 1. X-ray diffraction patterns of ZnONPs/AgNPs promoters.

Plant Growth

Foliar application of ZnONPs and ZnONPs/AgNPs significantly enhanced vegetative growth in coffee seedlings. ZnONPs-only treatment led to a 58.2% increase in plant height (Table 1), consistent with studies in wheat and rice where ZnONPs stimulated phytohormone pathways and cell division (Raliya, Tarafdar, and Biswas, 2016; Doolette, Read, Howell, Cresswell, and Lombi, 2020). Zinc is essential for auxin biosynthesis and activates carbonic anhydrase and RNA polymerase, key enzymes in cell elongation (Marschner, 2012). Stem diameter did not differ significantly (Table 2), but trends suggest enhanced growth vigor, corroborating reports in maize and bean under similar nanoparticle exposure (Burklew, Ashlock, Winfrey, and Zhang, 2012; Siddiqi and Husen, 2017). On the other hand, the effect of ZnONPs has been documented as a result of Zn's role as a micronutrient in plants, regulating basic mechanisms such as germination, photosynthetic pigment synthesis, and cell elongation (Yuvaraj and Subramanian, 2020).

Table 1. Plant height and stem diameter of coffee seedlings treated with different concentrations of ZnONPs and ZnONPs/AgNPs in combination with fertilizer doses.

Treatments	Plant height	Stem diameter
	cm	mm
Control	9.83 c	2.68
Fertilizer	10.95 bc	2.84
ZnONPs+2.5AgNPs	12.21 b	3.11
ZnONPs+1.5AgNPs	12.70 b	3.06
ZnONPs+2.5AgNPs+Fertilizer	13.14 b	2.81
ZnONPs+1.5AgNPs+Fertilizer	13.16 b	3.06
ZnONPs+Fertilizer	14.11 ab	2.80
ZnONPs	15.55 a	3.06
Standard error	0.22 *	0.04 ns

Means with different letters in the columns indicate that there are no significant differences ($p \leq 0.05$). *: Significant differences ($p \leq 0.05$). ns: no significant difference.

Table 2. Leaves number, unit leaf area, and total chlorophyll content of coffee seedlings treated with different concentrations of ZnONPs and ZnONPs/AgNPs in combination with fertilizer doses.

Treatments	Leaves number	Unit leaf area	Total chlorophyll content
		cm^2	mmol m^{-2}
Control	11.50 e	23.15	290.64 e
Fertilizer	13.25 c	25.53	393.54 c
ZnONPs+2.5AgNPs	14.50 b	31.46	325.94 d
ZnONPs+1.5AgNPs	14.00 c	28.43	522.60 b
ZnONPs+2.5AgNPs+Fertilizer	13.75 c	27.68	528.41 a
ZnONPs+1.5AgNPs+Fertertilizer	13.13 d	25.82	474.08 b
ZnONPs+Fertilizer	13.75 c	27.08	491.60 b
ZnONPs	14.50 a	36.70	439.33 c
Standard error	1.00	1.01 ns	13.12

Means with different letters in the columns indicate that there are no significant differences ($p \leq 0.05$). *: Significant differences ($p \leq 0.05$). ns = no significant difference.

Leaf Growth and Total Chlorophyll Concentration

Leaf number emitted per plant was significantly higher in the ZnONPs (Table 2), with an increase over the control treatment of 26.09%. These results indicate that plants treated with ZnONPs+2.5AgNPs plus mineral fertilizers consequently increase their photosynthetic potential, although such conjectures should be made carefully, suggesting further measurements such as CO_2 assimilation rates or photosystem II activity through chlorophyll fluorescence techniques. Total chlorophyll content was higher in the ZnONPs+2.5AgNPs + Fertilizer treatment, which is consistent with those obtained by other authors who found in peanut and tomato plants, increases in chlorophyll synthesis and protection of the photosynthetic apparatus (Prasad *et al*., 2012; Lahiani *et al*., 2013). This is most likely due to the enhancement produced by Zn in the ultrastructure of chloroplasts, protecting the thylakoid membranes, especially under stress conditions. While Ag modulates reactive oxygen species (ROS) synthesis pathways to stabilize photosynthetic pigments (Sing *et al*., 2018). On the other hand, Nair *et al*. (2010) found that increasing leaf area and chlorophyll content influences biomass production, causing plants to be able to resist and recover from stress situations. Similarly, Fortis-Hernández *et al*. (2024) reported that foliar application of ZnONPs increased the size and accumulation of biomass in lettuce plants; in addition, regardless of the concentration applied, there was an increase in the concentration of phytochemical compounds, vitamin C and antioxidant capacity, as well as the accumulation of Zn in the internal leaf tissue of lettuce, being more evident in the treatments with higher concentration of ZnONPs.

Functional Groups

Infrared spectra obtained from leaf, stem and root samples showed patterns that are consistent with those previously reported in coffee plants (Wang, Fu, and Lim, 2011; Wang and Lim, 2012). Functional groups were identified throughout the plant related to caffeine, proteins, water and carbohydrates, similar to those reported by other authors (Ribeiro, Ferreira, and Salva, 2011; Amir *et al*., 2013; Barrios, Collazos, and Gutiérrez, 2021), although the transmittance intensity of the spectra varied in the different plant organs (Figures 2a, 2b and 2c).

O-H vibrations were observed at 3318 cm^{-1} corresponding to water (Amir *et al*., 2013), as well as moisture content in plant tissues. The most intense vibrations were recorded in the leaves, stems, and roots of plants treated with ZnONPs+1.5AgNPs+Fertilizer, ZnONPs+1.5AgNPs, and ZnONPs+2.5AgNPs+Fertilizer (Figure 2). However, low water activity was recorded in the stems and roots of plants treated with ZnONPs+1.5AgNPs+Fertilizer, suggesting that the effect of nanofertilizers varies with the area of application and could have limited mobility in some plant organs. In contrast, treatments with ZnONPs+1.5AgNPs and ZnONPs+2.5AgNPs+Fertilizer revealed higher water activity in all plant parts.

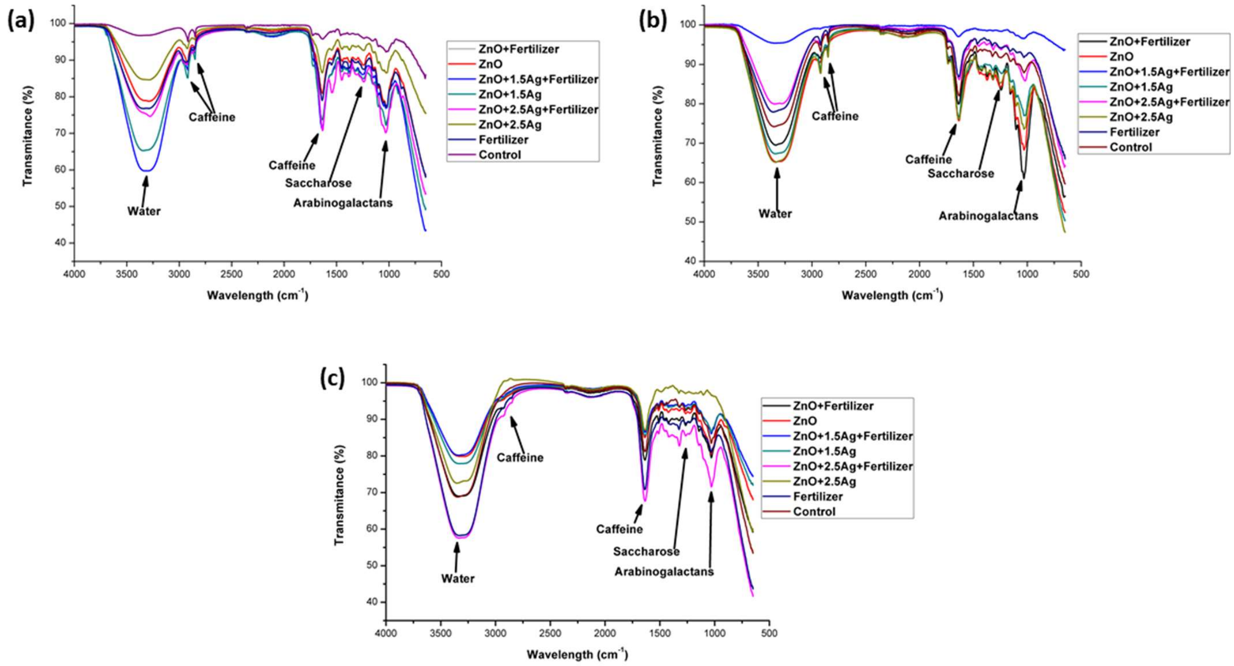

Figure 2. FT-IR spectra measured in the coffee plants: (a) leaves, (b) stem, and (c) roots.

The identification of caffeine with peaks in the stem spectra between 2923 and 2851 cm^{-1} agrees with reports by other authors that revealed the presence of caffeine in the range of 1650 and 1600 cm^{-1} (Reis, Franca, and Oliveira, 2013; Craig, Botelho, Oliveira, and Franca, 2018) (Figure 2). The bands identified at 1450 and 1000 cm^{-1} are related to chlorogenic acids, carbohydrates, and proteins (Navarini *et al*., 1999; Lyman, Benck, Dell, Merle, and Murray, 2003), and specifically the band at 1736 cm^{-1} is more characteristic of chlorogenic acid (Badmos, Lee, and Kuhnert, 2019). The highest intensities recorded and related to chlorogenic acid, carbohydrates, and proteins were higher in the leaves and roots of plants treated with ZnONPs+2.5AgNPs+Fertilizer, while in the stem of plants treated with ZnONPs, such activity was higher. On the contrary, in the plants of the control treatment, a lower intensity was recorded in these same bands (Figure 2). The peaks recorded in the range of 1248 and 1052 cm^{-1} correspond to sucrose (Craig *et al*., 2018; Munyendo, Njoroge, and Hitzmann, 2022), while the observed peak 1021 cm^{-1} is related to arabinogalactans. The carbohydrate groups were recorded in the region of 1500 to 800 cm^{-1}. In contrast, the control group showed lower intensity in these same bands (Figure 2).

The more intense absorbance bands related to caffeine, sucrose, and polysaccharides (Figure 2) indicate regulated secondary metabolism and biochemical adjustments under ZnONPs/AgNPs nanoparticle exposure. The increase of C-O (1080 cm^{-1}) and amide I (1640 cm^{-1}) bands in ZnONPs/AgNPs treatments reveals more intense protein and carbohydrate metabolism. Similar metabolic activation has been linked to ZnONPs elicitation in plants (Nair *et al*., 2010; Ma *et al*., 2010b). This biochemical modulation reflects a plant response under stress conditions and increased nutrient transport in nanofertilizer-treated plants (Agarwal, Kumar, and Rajeshkumar, 2017).

Although the effects of ZnONPs/AgNPs on vegetative growth are evident, especially on plant height and leaf emission, as well as on chlorophyll concentration and the synthesis of functional groups with slight variations in leaves, stem, and roots, the study is limited to the early stages of ontogeny of coffee plants. This is why extrapolating results to other phenological stages of the crop requires caution, as recent studies have shown the need to evaluate both the persistence of nanoparticles and their uptake kinetics, as well as possible toxic effects on plants at all phenological stages (Raliya and Tarafdar, 2013).

CONCLUSIONS

The crystalline nature of the analyzed nanoparticles was demonstrated, which showed sharp peaks consistent with the hexagonal wurtzite structure. In contrast, the AgNPs showed structures corresponding to the cubic phase of metallic silver. Foliar application of ZnONPs significantly increased height and stimulated greater leaf emission in coffee plants.

The combination of ZnONPs+2.5AgNPs plus fertilizers increased the concentration of total chlorophylls, as well as the greater accumulation of caffeine, sucrose, and carbohydrates.

These results demonstrate the potential of nanofertilizers as growth promoters and modulators of functional metabolism in coffee seedlings, although direct parameters of productivity and bean quality were not evaluated.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests concerning this study.

FINANCING

Not applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: V.P.M., and L.A.R.L.; methodology: V.P.M., D.S.S.; validation: L.A.R.L., J.F.P.A. and M.A.S.M.; formal analysis: L.A.R.L., V.P.M., and D.S.S.; investigation: V.P.M., D.S.S., P.D.O.C., and L.A.R.L.; resources: J.F.P.A., L.A.R.L., and V.P.M.; data curation: V.P.M., D.S.S., and P.D.O.C.; writing-original draft preparation: V.P.M., D.S.S., P.D.O.C., L.A.R.L., J.F.P.A., and M.A.S.M.; writing-review and editing: V.P.M., L.A.R.L., and D.S.S.; visualization: J.F.P.A., and M.A.S.M.; supervision: L.A.R.L., J.F.P.A., W.O.C.M. and M.A.S.M.

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