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Enhancing tomato (*Solanum lycopersicum* Mill.) plant growth and rhizosphere microbiome with carbon nanodots and mycorrhizal fungi: Impact on root colonization, plant growth, chlorophyll, and nitrogen content**

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Abstract. This study investigated the effects of glycine-based (Gly-CNDs) and curcumin-based (Cur-CNDs) carbon nanodots, in combination with mycorrhizal fungi (MF), on the growth and rhizosphere microbiome of tomato plants (*Solanum lycopersicum* Mill.). The experiment, conducted in 2024 in a controlled greenhouse environment, evaluated root colonization, plant height, stem thickness, chlorophyll content, nitrogen uptake, and microbial abundance in the rhizosphere. Mycorrhizal inoculation significantly enhanced root colonization, particularly when combined with nanodots, improving nutrient uptake and chlorophyll content. The application of Gly-CNDs and Cur-CNDs, both independently and synergistically with MF, positively influenced root biomass, chlorophyll *a* and *b* content, and nitrogen accumulation in leaves. Microbial profiling showed that treatments increased bacterial populations (notably *Bacillus* spp. and *Pseudomonas* spp.) while suppressing fungal abundance in the rhizosphere, indicating a shift in microbial community structure. Among all treatments, the combination of mycorrhizal fungi with Gly-CNDs (MF+NSG) proved most effective in promoting plant growth and health status. These findings highlight the potential of CNDs as sustainable biostimulants to enhance tomato production, improve soil microbiome dynamics, and reduce reliance on chemical fertilizers.

Keywords: plant growth, tomato, carbon nanodots, mucorrhizal fungi, rhizosphere microbiome

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is a commercially and nutritionally important crop. However, its production is often constrained by abiotic and biotic stresses, declining soil microbiome diversity, and nutrient limitations (Qi *et al.,* 2022; Cirillo *et al.,* 2023; Ouyang *et al.,* 2023; Turan *et al.,* 2023). Therefore, there is an urgent need for alternative sustainable technologies to enhance tomato production and protect it from phytopathogens (Xu *et al.,* 2024).

Conventional agricultural practices that rely heavily on synthetic pesticides and fertilizers to improve crop production have significant negative environmental impacts. The use of synthetic fertilizers contributes to the degradation of soil biodiversity by limiting the role of nitrogen-fixing bacteria, thereby intensifying the decomposition of organic matter and humus. The decrease in organic matter content also changes the physical structure of the soil. These alterations lead to the modulation of various interconnected soil physiological processes (Tripathi *et al.,* 2020). Moreover, reduced enzymatic activity and disrupted nutrient cycling, particularly of nitrogen and phosphorus, impair

the soil's self-regulatory and regenerative capacities (Chen *et al.,* 2021). Therefore, unjustified and prolonged use of chemical fertilizers exerts negative environmental effects, leading to soil acidification and altered structure of bacterial and fungal communities (Bai *et al*., 2020; Zhang *et al*., 2022). Acidification also affects nutrient bioavailability and may increase heavy metal toxicity (Rengel, 2011). In conventional agriculture, the widespread use of chemical fertilizers results in their leaching into nearby rivers, posing a major threat to global soil biodiversity (Hui *et al.,* 2022). Moreover, their accumulation in aquatic environments can lead to eutrophication and disrupt the functioning of aquatic ecosystems (Vitousek *et al.,* 2009). Organic nanoparticles and mycorrhizal fungi can become a sustainable alternative to chemical fertilizers, as they improve soil structure, its microbiome, crop quality, and contribute to environmental sustainability (Vázquez *et al.,* 2000; Mącik *et al.,* 2020). Arbuscular mycorrhizal fungi (AMF) help reduce plant stress, increase plant productivity, lower agrochemical use, and decrease environmental pollution (Zadehbagheri *et al.,* 2014; Wang *et al.,* 2020; Jamiołkowska *et al.,* 2021). Mycorrhizal fungi have the potential to become an important biotechnological tool for restoring agricultural ecosystems. They protect plants from soil-borne diseases, facilitate nutrient uptake (especially of less assimilable nutrients), improve soil aggregation, enhance drought stress resistance, and contribute to the increased biodiversity of the soil microbiome (Whipps, 2004; Zadehbagheri *et al.,* 2014; Jamiołkowska *et al.,* 2020a, 2021; Wang *et al.,* 2025).

Nanotechnology has emerged as a transformative approach for sustainable agriculture, offering innovative solutions to enhance crop productivity while minimizing the environmental impact. Nanomaterials, ranging from 1 to 100 nm in size, have attracted significant research interest due to the unique combination of their properties. Carbon nanodots (CNDs) are ultrasmall carbon-based nanoparticles, typically less than 10 nanometers in size, known for their excellent fluorescence, high water solubility, biocompatibility, and low toxicity; hence, they are ideal compounds for agricultural applications (Joudeh *et al.,* 2022; Wu *et al*., 2023). CNDs generally exhibit a spherical or quasi-spherical morphology. They may be either amorphous or contain graphitic crystalline domains, as demonstrated by lattice fringes observed under high-resolution transmission electron microscopy. The carbon core is composed of sp^2 and sp^3 hybridized carbon atoms, contributing to their unique structure and physical behavior (Vejpravová, 2021). One of the most important physical characteristics of carbon dots is that their surfaces are decorated with various functional groups, *i.e*., hydroxyl (-OH), carboxyl (-COOH), and amino (-NH_2) groups (Cai *et al.,* 2025). These groups not only impart excellent water solubility and dispersibility in polar solvents but also play a crucial role in modulating surface charge and interaction with biological or environmental systems. CNDs typically exhibit a negative zeta potential due to the presence of surface carboxyl groups; however, this can be adjusted through appropriate surface modifications (Yahaya *et al.,* 2020).

CNDs demonstrate high thermal stability, generally decomposing at temperatures above 200°C, depending on their composition and surface functionalization (Rimal *et al.,* 2020). This thermal resilience makes them suitable for use in high-temperature environments. Although the mechanical properties of CNDs alone are not well-documented, their incorporation into composite materials like polymers has been shown to enhance tensile strength, flexibility, and overall mechanical performance. Overall, the physical properties of carbon dots, including their nanoscale size, surface functionality, structural variability, thermal robustness, and dispersibility, make them highly versatile materials for applications in sensing, imaging, drug delivery, and agriculture (Qureshi *et al*., 2024).

Owing to their surface functional groups, CNDs are easily modified and have wide-ranging applications. In agriculture, they promote plant growth, enhance nutrient uptake, and help manage plant pathogens (Shoala, 2020; Prokisch *et al.,* 2024).

Carbon nanoparticles (CNDs) are also used in agriculture as seed germination enhancers and plant stress alleviators (Guo *et al.,* 2022). The positive effect of CNDs on various plants indicates their high potential for agricultural applications, playing a significant role in promoting crop growth and improving the sustainability of agricultural production (Li G. *et al.,* 2023; Abinaya *et al.,* 2024). The primary pathways for CD uptake in plants include root absorption from soil/water and foliar absorption. Research demonstrates that CDs can penetrate plant cells and be transported with water and minerals from roots to stems and leaves. They enter through cell walls and plasmodesmata via extracellular pathways in intercellular spaces and the apoplast, and then move through the cortex into the xylem via symplastic transport (Li *et al.,* 2018, 2021; Yan *et al.,* 2021; Chen N. *et al.,* 2024).

Curcumin, a bioactive compound with well-documented antimicrobial properties, can protect plants against various pathogens, thereby enhancing resilience to biotic and abiotic stresses. Curcumin-functionalized carbon nanodots (Cur-CNDs) further amplify these benefits by improving uptake efficiency and distribution within plant tissues, leading to enhanced growth and stress tolerance (Anas *et al.,* 2024). Glycine, an essential amino acid, plays a crucial role in nitrogen metabolism and chlorophyll biosynthesis, making glycine-derived carbon nanodots (Gly-CNDs) promising candidates for enhancing nitrogen assimilation in plants (Li Y. *et al.,* 2023). Despite their potential, the effects of Cur-CNDs and Gly-CNDs on tomato growth, root-microbe interactions, and overall rhizosphere health remain largely unexplored.

This study aims to explore the role of curcumin carbon nanodots (Cur-CNDs), Glycine carbon nanodots (Gly-CNDs), and mycorrhizal fungi in promoting tomato plant growth, with a focus on root colonization, chlorophyll content, nitrogen uptake, and rhizospheric microbial diversity. The findings from this research will contribute to the development of nanotechnology-based bio-stimulants for sustainable tomato cultivation, reducing reliance on chemical inputs while enhancing plant health and soil fertility.

2. MATERIALS AND METHODS

2.1. Pot experiment

The studies were conducted in 2024 at the greenhouse of the Felin Experimental Station, University of Life Sciences in Lublin, Poland. The experiments were performed on tomato (*Solanum lycopersicum* Mill.) plants of the variety 'Lubań' (bred by PNOS Sp. z o.o., Ożarów Mazowiecki, Poland) inoculated with a mixture of mycorrhizal fungi and watered with curcumin carbon nanodots (Cur-CNDs, Gly-CNDs). The mycorrhizal inoculum ENDOMIX MYKOFLOR (Mykoflor, Końskowola, Polska) consisted of a mixture of spores and mycelial fragments of mycorrhizal fungi (*Rhizophagus intraradices*, *Funneliformis mosseae*, *Glomus claroideum*, *Entrophospora claroidea, Gigaspora margarita*, and *Entrophospora* sp.) suspended in horticultural substrate (10^4 CFU L^{-1} g^{-1} soil).

Tomato seedlings used in the experiment were produced at the Felin Experimental Station, University of Life Sciences in Lublin (Poland), following the standards established for the production of plant seedlings from the family *Solanaceae*. Three-week-old tomato seedlings (developmental stage BBCH 103 – three fully developed true leaves) were planted in the second decade of May into 1 L plastic containers filled with universal horticultural substrate with pH 6.5 (Kronen, Lasland Sp. z o.o., Cerkwica, Poland). Before planting, 0.56 g of dry mycorrhizal fungi inoculum (ENDOMIX MYKOFLOR) was applied to the substrate of each plant. Four weeks after setting the experiment (BBCH 107), the plants were watered with curcumin carbon nanodots (Cur-CNDs) and glycine carbon nanodots (Gly-CNDs) (1.1 mL of carbon nanodots/100 mL of water) at a rate of 25 mL of the mixture per plant according to the experimental treatments described in Table 1. For each experimental combination, 20 plants were prepared (5 plants in four replicates). During the experiment, the plants were grown under natural greenhouse lighting. The average temperature in the greenhouse during plant growth was 23°C, and humidity ranged from 27 to 35%. The greenhouse was automatically ventilated to ensure optimal temperature and humidity. The plants were placed on flood tables lined with a potting mat, which was automatically flooded once a day for 2 min. During the experiment, the plants were not fertilized.

Table 1. Experimental treatments

Symbol	Description of experimental treatment
C	Control plants
MF	Mycorrhizal plants
Cur-CNDs	Plants watered with Curcumin carbon nanodots
Gly-CNDs	Plants watered with Glycine carbon nanodots
MF+ Cur-CNDs	Mycorrhizal plants watered with Curcumin carbon nanodots
MF+ Gly-CNDs	Mycorrhizal plants watered with Glycine carbon nanodots

2.2. Synthesis of carbon nanodots of Glycine and Curcumin

The carbon nanodots (CNDs) were synthesized using pyrolysis. For the preparation of Glycine carbon nanodots (Gly-CNDs), glucose and glycine were mixed thoroughly in a solid powdered form at a mass ratio of 1:4. A mixture of 100 g of glucose and 400 g of glycine was then placed in an oven at 180°C for 2 h. During heating, carbonization of glucose occurred, leading to the formation of voluminous, low-density, sponge-like, black, and fragile material. The obtained solid was subsequently ground into powder and suspended in water. The suspension was then subjected to a two-step filtration process, initially passing through a coarse filter and subsequently through a fine filter to remove larger impurities. The resulting filtrate containing nanoparticles and only minimal amounts of residual starting materials was freeze-dried (lyophilized) and then powdered again. This final product was characterized as a brown-colored substance with a sweet taste, indicative of the presence of residual glycine or glucose derivatives (Fig. 1).

The preparation of curcumin-based carbon nanodots (Cur-CNDs) was carried out following the same pyrolysis procedure described previously. In this case, curcuma spice powder and glucose were thoroughly mixed in a solid powdered state at a 4:1 mass ratio (400 g of curcuma spice powder and 100 g of glucose). The resulting mixture was then subjected to heat treatment in an oven at 180°C for 2 h. This thermal process led to the carbonization of glucose, resulting in a voluminous, porous, dark-colored material.

a) b) c) d) e)

Fig. 1. Steps of Gly-CNDs preparation: a) mixture of glycine and glucose, b) after heat treatment of the mixture, c) extraction with water, d) freeze-dried material before grinding, e) the powdered Gly-CNDs.

Following thermal treatment, the obtained material was ground into a fine powder and then extracted with distilled water. The suspension was filtered sequentially through coarse and fine filters to remove larger particles and impurities. The resulting filtrate containing primarily Cur-CNDs along with minimal residual substances was subjected to freeze-drying (lyophilization). After lyophilization, the product was ground again to obtain a fine powder. The final powdered Cur-CNDs material exhibited excellent water solubility and demonstrated strong fluorescence properties, making it suitable for potential applications in biological imaging and analytical sensing.

2.3. Assessment of root colonization by arbuscular mycorrhizal fungi

Root samples were collected in plastic containers after two months of plant growth to analyze the degree of root colonization by mycorrhizal fungi. The roots were stained using the method developed at the Department of Microbiology, Research Institute of Horticulture (Derkowska *et al*., 2015). Microscope slides were prepared from stained roots by selecting 30 fragments, each approximately 1 cm long. The fragments were arranged in parallel on a microscope slide containing glycerin and then gently squashed with a cover slip. Histological slides were analyzed using a Nikon 50i microscope (objectives: 20x, 40x, 60x, 100x magnification). Photographic documentation of the observed mycorrhizal structures was collected. The degree of tomato root colonization by mycorrhizal fungi was assessed using the Trouvelot method (1986). Based on the results, mycorrhizal frequency (F%) and root colonization intensity (M%, m%) were calculated using the MYCOCALC software available at: http://www2.dijon.inra.fr/mychintec-/Mycocalcprg/download.html.

2.4. Measurements of plant height and stem thickness

The stem height and thickness of tomato plants were measured at the beginning of flowering (BBCH 62). Ten plants from each experimental treatment were randomly selected to measure the height of the aerial part of the plant (from the ground level to the apex) and the thickness of the stem base. Stem thickness was measured using an electronic caliper (Limit CDN-NT IP67). The length and thickness of the stem (cm) were recorded as the average values obtained from measurements conducted within each experimental treatment.

2.5. Root fresh and dry weight analysis

Assessment of fresh and dry root weight was carried out four weeks after the application of the mycorrhizal inoculum and nanoparticles, corresponding to developmental stage BBCH 62 (beginning of flowering). These parameters were evaluated for 10 plants from each experimental treatment. Fresh root weight was expressed in g fw. The plant material was dried in a ventilated room at 23-25°C for 7 days. It was subsequently weighed, and the results were expressed in g dw.

2.6. Leaf greenness index (SPAD) assessment

Leaf greenness was assessed on 10 plants from each experimental treatment by measuring the third fully developed leaf from the top of the plant. Leaf greenness was assessed six weeks after the application of mycorrhizal fungi and two weeks after the application of nanoparticles using a SPAD-502 chlorophyll meter (Konica Minolta). Each experimental treatment included 10 measurement points in total. SPAD values were averaged for each experimental treatment.

2.7. Analysis of leaf chlorophyll content

Three weeks after mycorrhizal inoculation and nanoparticle application to the plant rhizosphere, the chlorophyll *a*, *b*, and *a* + *b* contents in the leaves were analyzed following the methodology described by Kursa *et al*. (2024). From each experimental combination, 0.2 g of leaves was weighed. Measurements were taken at the following wavelengths: 663 nm for chlorophyll *a*, 645 nm for chlorophyll *b*, and 652 nm for total chlorophyll (*a* + *b*). The pigment content (mg g^{-1} fw) was then calculated according to the following formulas:

$$Chlorophyll\ a = \frac{(12.7D663 - 2.7D645)V}{1000\ m}, \tag{1}$$

$$Chlorophyll\ b = \frac{(22.9D645 - 4.7D663)V}{1000\ m}, \tag{2}$$

$$Chlorophyll\ a + b = \frac{27.8D652V}{1000\ m}, \tag{3}$$

where: D − absorbance at a given wavelength, V − total volume of extract (cm^3), m − sample mass (g).

2.8. Leaf total nitrogen analysis

To measure leaf nitrogen content, tomato leaf samples were collected at the beginning of flowering (BBCH 62). The third fully developed leaf from the top of the shoot was selected for laboratory analysis. Ten leaves were randomly selected from each experimental treatment. Nitrogen content was determined using the Kjeldahl method (PN-EN ISO 5983-1:2006) with a Kjeltec 2300 apparatus (FOSS, Sweden) following prior mineralization with H_2SO_4 in a Tecator Digestor Auto 20 system (FOSS, Sweden).

2.9. Analysis of microbial community abundance in the rhizosphere

The study was conducted at the beginning of flowering (BBCH 62). Rhizosphere soils were collected from individual experimental treatments, and microbiological analyses were performed following the method described

by Jamiołkowska *et al.* (2020a). Rhizosphere soil samples were collected from 5 plants in each experimental treatment. Soil samples from the same experimental treatments were combined in laboratory conditions, divided into 10 g weighed amounts, and prepared for further analysis (4 replicates per experimental treatment).

Soil solutions were prepared in laboratory conditions by diluting 10 g of soil from each sample in a serial dilution range from 10^{-1} to 10^{-7}. The total bacterial population was determined on Petri dishes using nutrient agar medium (Difco). The population of *Bacillus* sp. was determined using tryptic soy agar (Difco), while the *Pseudomonas* sp. population was assessed on *Pseudomonas* agar F (Difco). For *Bacillus* spp. isolation, soil dilutions were heated for 20 min at 80°C (Jamiołkowska *et al.,* 2020a). Martin's medium was used to determine the fungal population in the rhizosphere (Patkowska and Krawiec, 2016). Petri dishes were stored at 24°C in the dark for 2-7 days. After incubation, the number of microorganisms was determined and expressed as CFU g^{-1} soil dw (Colony Forming Units/g soil dry weight). Fungal colonies grown from the rhizosphere soil were measured and subsequently transferred to potato dextrose agar (Difco) slants. The isolated fungi were transferred onto specialized culture media, including potato dextrose agar, selective nutrient agar, Czapek-Dox, and malt medium, and identified to the species level using available keys and monographs as described by Patkowska and Krawiec (2016).

2.10. Statistical analysis

The data were analyzed using Statistica software version 13.3 (1984-2017 TIBCO Software Inc., Palo Alto, CA, USA). A one-way analysis of variance (ANOVA) was conducted, and the significance of differences was assessed using Tukey's post hoc test and Kruskal-Wallis test at a significance level of $p = 0.05$.

3. RESULTS

3.1. Degree of root colonization by mycorrhizal fungi

The laboratory analysis of the tomato roots showed that the MF treatment significantly increased the abundance of arbuscular mycorrhizal fungi in the roots, with the highest colonization observed in the MF treatment (30%) (Table 2). It was also observed that the roots of plants watered with Gly-CNDs showed the highest relative and absolute mycorrhizal intensity in the tomato roots, *i.e.*, 2.88 and 10.73%, respectively (Table 2, Fig. 2).

3.2. Stem height and thickness

The study showed that the mycorrhizal plants were significantly shorter (by 26.7%) than the control plants. The tallest plants were recorded after the application of curcumin carbon nanodots (Cur-CNDs). These plants were significantly taller only compared to the mycorrhizal plants

Table 2. Effect of mycorrhizal inoculum and nanoparticles on the degree of root colonization by arbuscular mycorrhizal fungi in tomato plants

Experimental treatment	F	M	m
	(%)		
C	18.89b	1.70ab	8.92ab
MF	30.0a	2.59a	8.68ab
Cur-CNDs	17.78bc	1.37ab	7.72b
Gly-CNDs	26.67ab	2.88a	10.73a
MF+ Cur-CNDs	13.33c	1.28ab	9.88ab
MF+ Gly-CNDs	27.78ab	2.24a	7.95b

C – control, MF – plants inoculated with mycorrhizal inoculum; Cur-CNDs – plants watered with Curcumin carbon nanodots, Gly-CNDs – plants watered with Glycine carbon nanodots, MF+ Cur-CNDs – plants inoculated with mycorrhizal fungi and watered with Curcumin carbon nanodots, MF+ Gly-CNDs – plants inoculated with mycorrhizal fungi and watered with Glycine carbon nanodots, F – mycorrhizal frequency, M – relative mycorrhizal intensity (refers to the entire sample), m – absolute mycorrhizal intensity (refers to fragments where any mycorrhizal colonization occurred). a, b, c – values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

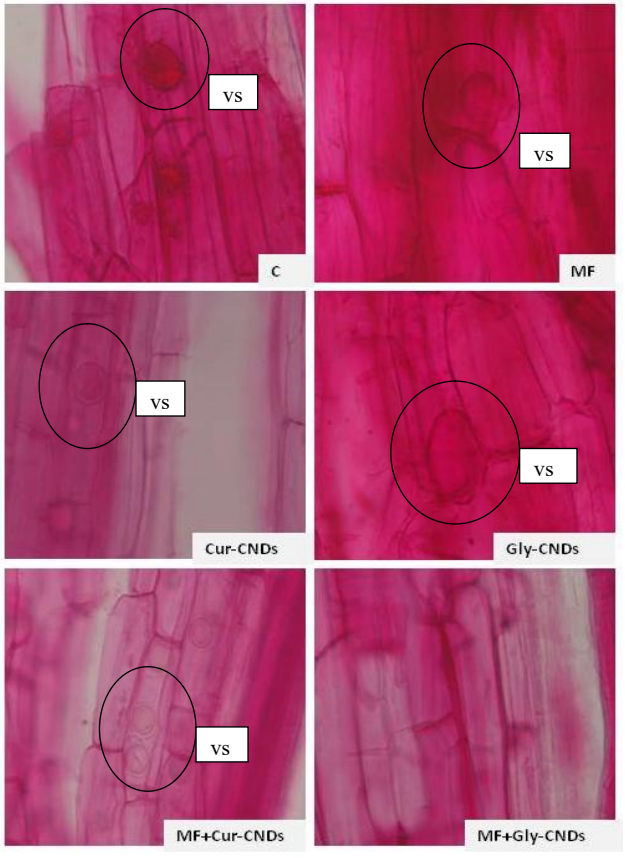

Fig. 2. Arbuscular mycorrhizal fungi (MF) structures in the roots of tomato plants. vs – vesicles; microscope lens magnification 40x. Other explanations as in Table 2.

Table 3. Selected biometric characteristics of tomato plants in the experimental treatments (average ± SD)

Experimental treatment	Stem height (cm)	Stem base thickness (mm)	Fresh root weight (g)	Dry root weight (g)
C	87.00±13.81a	88.00±8.37a	29.64±4.76c	3.89±0.70b
MF	63.80±9.26b	90.00±7.07a	34.54±9.56bc	5.01±1.27ab
Cur-CNDs	90.00±14.38a	94.00±5.48a	50.07±8.79a	6.01±0.24ab
Gly-CNDs	77.00±11.21ab	94.00±5.48a	42.24±9.35abc	5.76±1.05ab
MF+ Cur-CNDs	80.30±8.90ab	94.00±5.48a	49.59±8.04ab	6.35±1.34ab
MF+ Gly-CNDs	82.40±13.96ab	96.00±5.48a	50.15±5.78a	6.90±0.89a

C – control, MF – plants inoculated with mycorrhizal fungi, Cur-CNDs – plants watered with Curcumin carbon nanodots, Gly-CNDs – plants watered with Glycine carbon nanodots, MF+ Cur-CNDs – plants inoculated with mycorrhizal fungi and watered with Curcumin carbon nanodots, MF+ Gly-CNDs – plants inoculated with mycorrhizal fungi and watered with Glycine carbon nanodots. a, b, c – values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

Table 4. SPAD greenness index and chlorophyll content in wheat leaves after spraying seedlings with plant extracts

Experimental combination	SPAD index ±SD	Chlorophyll *a* ± SD	Chlorophyll *b* ± SD	Chlorophyll *a*+*b* ± SD
		(mg g^{-1} fw)		
C	48.54±7.64a	2.44±0.14c	0.77±0.19c	3.17±0.35b
MF	44.06±2.98a	2.74±0.19b	1.38±0.46b	4.79±0.99a
Cur-CNDs	53.13±2.53a	2.45±0.03bc	1.05±0.06bc	3.39±0.02b
Gly-CNDs	45.59±5.89a	2.47±0.04bc	1.19±0.10bc	3.53±0.03b
MF+ Cur-CNDs	45.67±4.33a	3.17±0.10a	2.09±0.03a	5.25±0.04a
MF+ Gly-CNDs	44.06±2.98a	3.06±0.05a	1.27±0.04bc	4.32±0.02ab

Explanations as in Table 3.

(MF) (an increase of 41.1%), but no significant differences were noted compared to plants from the other experimental combinations, although the plants showed a better growth tendency after the application of Cur-CNDs (Table 3). The thickest stem base was recorded in the mycorrhizal plants and those watered with glycine carbon nanodots (MF+Gly-CNDs), but this value did not differ significantly from the other experimental combinations (Table 3). Nevertheless, it is worth noting that the application of the mycorrhizal inoculum combined with curcumin and glycine carbon nanodots had a favorable effect on the stem base thickness compared to the control or the treatment with the mycorrhizal inoculum alone (Table 3).

3.3. Fresh and dry root weight

The highest fresh root weight was observed in plants treated with mycorrhizal fungi combined with glycine carbon nanodots (MF+Gly-CNDs), with an increase of 69.2% compared to the control, and in plants treated with curcumin carbon nanodots (Cur-CNDs), where the observed increase in fresh root weight was 68.9% higher than in the control. The fresh root weight after the MF+Gly-CNDs and Cur-CNDs application was significantly higher than the fresh weight of mycorrhizal roots (MF) (by 45.2 and 45%, respectively) (Table 3). The results indicated that the combination of glycine nanodots with the mycorrhizal inoculum (MF + Gly-CNDs) as well as the use of Cur-CNDs nanodots separately contributed to a significant increase in the fresh root weight. The highest dry root weight was recorded for mycorrhized roots treated with glycine nanodots (MF + Gly-CNDs), with a significant increase by 77.4% compared to the control (C). The dry root weight from the other experimental combinations was higher than in the case of the control, although the differences were not statistically significant. However, the obtained results indicate an upward trend in the tested trait after the application of MF and carbon nanodots (Table 3).

3.4. Leaf greenness index and chlorophyll content

The highest SPAD index was recorded for the leaves of plants watered with curcumin nanodots (Cur-CNDs), and its value was only 9.5% higher than the SPAD for the mycorrhizal plants (MF), which was the lowest among the experimental combinations tested. The results did not differ significantly (Table 4).

The tomato leaves exhibited varying levels of plant pigments (chlorophyll *a*, chlorophyll *b*, and total chlorophyll *a* + *b*), depending on the experimental treatment used (Table 4). The highest chlorophyll *a* content was recorded in the leaves of plants treated with the mycorrhizal inoculum combined with carbon nanoparticles (MF+ Cur-CNDs, MF+ Gly-CNDs) and differed significantly from the other

experimental combinations. However, the highest increase in the chlorophyll *a* content was recorded for the combinations MF+ Cur-CNDs and MF + Gly-CNDs compared to the control (by 29.9 and 25.4%, respectively). The highest chlorophyll *b* content was observed in the leaves of plants treated with mycorrhizal fungi and curcumin nanodots MF+Cur-CNDs (171.4% increase compared to the control) and in combination with the mycorrhizal inoculum (79.2% increase compared to the control). These values differed significantly from the control. The highest content of total chlorophyll *a* + *b* was found in the leaves of plants treated with the mycorrhizal inoculum (MF) and watered with MF+ Cur-CNDs. In the case of chlorophyll *a* + *b* (except for MF and MF+ Gly-CNDs), statistically significant differences were observed compared to the control group and the other experimental fertilizations.

3.5. Leaf total nitrogen content

The study demonstrated a beneficial effect of the mycorrhizal inoculum combined with nanoparticles on the nitrogen content in the tomato leaf samples. In samples in which mycorrhizae were applied with carbon nanoparticles (MF+ Cur-CNDs), a 9.9% increase in the nitrogen content was observed compared to the control samples. These differences were statistically significant. In the case of samples in which mycorrhizae were applied with glycine nanoparticles (MF+ Gly-CNDs), a 6.4% increase in the tested parameter was also observed compared to the control samples. Significantly lower nitrogen content was observed in the leaf samples from plants watered solely with glycine nanoparticles (Gly-CNDs), carbon nanoparticles (Cur-CNDs), and inoculated with the mycorrhizal inoculum (MF) (Table 5).

Table 5. Total nitrogen content (%) in tomato leaves

Experimental combination	Total nitrogen content (%) ±SD
C	1.71±0.11b
MF	1.49±0.09c
Cur-CNDs	1.51±0.13c
Gly-CNDs	1.42±0.13c
MF+ Cur-CNDs	1.88±0.16a
MF+ Gly-CNDs	1.82±0.16ab

Explanations as in Table 3.

3.6. Microbial community abundance in the rhizosphere

The quantitative and qualitative composition of rhizospheric microorganisms determined by the microbiological soil analysis showed significant variability. The mycorrhization, Cur-CNDs, and Gly-CNDs stimulated the growth of bacteria, including *Bacillus* sp. and *Pseudomonas* sp., in the tomato rhizosphere. Their population was statistically significantly higher than in the control. Depending on the experimental variant, the average bacterial population ranged from 15.33×10^6 to 95.6×10^6 CFU g^{-1} soil dw (Table 6). The most abundant total bacterial population was observed in the rhizosphere after the application of mycorrhizal fungi, curcumin carbon nanodots, or glycine carbon nanodots (averaging 95.66×10^6 and 84.66×10^6 CFU/g soil dw, respectively). A slightly less abundant total bacterial population was found after the mycorrhization of tomato plants (averaging 54.33 x 10^6 CFU/g soil dw) and after the application of only Cur-CNDs or Gly-CNDs (averaging 41.66×10^6 and 33.66×10^6 CFU g^{-1} soil dw). The lowest abundance of the bacterial population was observed in the rhizosphere of the control plants (15.33×10^6 CFU g^{-1} soil dw). A similar pattern was observed for *Bacillus* sp. and *Pseudomonas* sp. populations. The population abundance of *Bacillus* sp. ranged from 4.33×10^6 to 42.00×10^6 CFU g^{-1} soil dw, while the *Pseudomonas* sp. population varied from 8.66×10^6 to 53.00×10^6 CFU g^{-1} soil dw. An entirely opposite relationship was observed in the fungal population. The highest abundance of the fungal population was found in the rhizosphere of the control plants (96.33×10^3 CFU g^{-1} soil dw), while the lowest abundance was observed after the inoculation of mycorrhizal fungi and Cur-CSNDs (13.00×10^3 CFU g^{-1} soil dw). A small rhizospheric fun-

Table 6. Number of bacteria and fungi isolated from soil in individual experimental treatments

Experimental treatment	Total CFU – Bacteria (10^6 g^{-1} soil dw)	*Bacillus* sp. CFU (10^6 g^{-1} soil dw)	*Pseudomonas* sp. CFU (10^6 g^{-1} soil dw)	Total CFU – Fungi (10^3 g^{-1} soil dw)
Control	15.33f	4.33d	8.66d	96.33a
MF	54.33c	22.33c	28.33bc	44.33d
Cur-CNDs	41.66d	17.00c	23.00c	54.33c
Gly-CNDs	33.66e	13.00cd	18.33c	63.66b
MF+ Cur-CNDs	95.66a	42.00a	53.00a	13.00f
MF+ Gly-CNDs	84.66b	36.00b	45.00b	21.00e

Explanations as in Table 3.

gal population was also observed following the application of mycorrhizal fungi and glycine carbon nanodots (21.00 × 10^3 CFU g^{-1} soil dw). In the experimental combinations where the mycorrhizal inoculation was not used and only Cur-CNDs or Gly-CNDs were applied, the fungal population was slightly more abundant (on average 54.33 × 10^3 and 63.66 × 10^3 CFU g^{-1} soil dw, respectively), but still less abundant and significantly different from the control.

4. DISCUSSION

Global research indicates that farmers are becoming increasingly aware of the need to reduce the use of chemicals in agricultural production and replace them with organic alternatives (Sas-Paszt *et al.,* 2011, 2020; Jamiołkowska *et al.,* 2020b). The effectiveness of plant growth stimulants and improved fruiting can be enhanced using mycorrhizal fungi and organic nanoparticles, even replacing mineral fertilization (Li *et al.,* 2023).

Mycorrhizal fungi form symbiotic associations with plant roots, facilitating the uptake of essential nutrients, such as nitrogen and phosphorus, while receiving carbon from the host plant in return, which stimulates plant growth and yield (Martin, 2024). Carbon nanoparticles also promote plant development by improving absorption, ensuring more precise delivery to the plant, and minimizing losses caused by leaching (Satya *et al.,* 2024). While both CNDs and mycorrhizal fungi individually contribute to plant health, their combined efficacy, particularly concerning tomato growth, mycorrhizal root colonization, photosynthesis, and nitrogen metabolism, remains largely uninvestigated.

Carbon nanoparticles and mycorrhizal fungi play a crucial role in plant growth and quality improvement by increasing their nutrient content, photosynthetic activity, and metabolism (Wang *et al.,* 2023; Jamiołkowska *et al.,* 2021). Our research confirms the assertions of the aforementioned authors. The study demonstrated that plants fertilized with curcumin carbon nanodots (Cur-CNDs) tended to growth better than either the control plants or the mycorrhized plants. The use of curcumin-based carbon nanodots (Cur-CND) and glycine-based carbon nanodots (Gly-CND) improved tomato stem thickness. Similar trends were observed when carbon nanodots were combined with the mycorrhizal inoculum. Both treatments resulted in better plant growth than the control and in the mycorrhizal inoculum alone variant. Nanoparticles are absorbed by plants through various pathways, depending on their type and size as well as plant species and growth stage. The contact between nanoparticles and plant roots occurs through the adsorption of molecules onto the root surface. Since root hairs can release chemicals, such as mucilage or organic acids, the root surface carries a negative charge. This makes positively charged nanoparticles more likely to accumulate in the root and be readily absorbed through the root surface (Lv *et al.,* 2019). The root cell wall contains small pores that may prevent the passage of large nanocharged particles (Perez-de-Luque, 2017). Other studies have shown that certain nanoparticles can disrupt the plasma membrane and induce the formation of new pores in the epidermal cell wall, facilitating the entry of larger-diameter nanoparticles. When nanoparticles penetrate plant tissue, they can be absorbed by plant cells through various pathways, such as ion channels, endocytosis, binding to membrane proteins, or physical damage (Yu *et al.,* 2024). Wang *et al.* (2023) have suggested that the improvement in plant biometric traits is related to the significant impact of nanoparticles on plant physiological activities. These reports were confirmed in the present study. It was shown that the application of carbon nanodots and mycorrhizal fungi significantly improved root fresh and dry weight, particularly when glycine carbon nanodots were combined with mycorrhizal fungi (MF+Gly-CNDs). The combined application of carbon nanodots with the mycorrhizal inoculum (MF+Cur-CNDs; MF+Gly-CNDs) significantly increased chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a*+*b*) content in tomato leaves compared to applications using only carbon nanodots or the mycorrhizal inoculum alone. Recent studies (Li *et al.,* 2024) have demonstrated that carbon nanoparticle application significantly increased chlorophyll accumulation in rice leaves. These findings suggest that carbon nanodots may promote plant growth by stimulating chlorophyll biosynthesis. This mechanism may be linked to their ability to improve photosynthetic efficiency by increasing light absorption and electron transport in chloroplasts (Li *et al.,* 2021). Chen L. *et al.* (2024) demonstrated that varying concentrations of carbon nanoparticles altered plant morphological characteristics, including leaf area, branching number, and green leaf count, all of which showed decreasing trends with a rising nanoparticle concentration. In contrast, plant height, stem diameter, and leaf number remained unchanged despite the application of carbon nanoparticles. Chen L. *et al.* (2024) demonstrated, however, that the exposure of *Ficus tikoua* plants to carbon nanoparticles had no significant effect on chlorophyll *a* (Chl *a*) levels. However, the total chlorophyll *b* (Chl b) content was significantly lower in plants exposed to carbon nanoparticles compared to the control samples. Meanwhile, the Chl *a*/*b* ratio was significantly higher in plants treated with carbon nanoparticles compared to the control samples. Similarly, the application of mycorrhizal fungi (MF) in the root zone of tomato plants significantly improved the physiological and morphological parameters of the cultivated plants, including increased chlorophyll content (Jamiołkowska *et al.,* 2020b; Manila and Nelson, 2014; Mekkaoui *et al.,* 2024). The soil application of carbon nanoparticles combined with the tomato mycorrhization also significantly increased the foliar nitrogen content, demonstrating markedly higher levels compared to all the other experimental treatments. The increased nitrogen

content in leaves represents a highly beneficial effect, as this element is essential for producing enzymes required for photosynthesis (Ahanger *et al.,* 2015). Nitrogen plays a key role in chlorophyll synthesis, protein production, nucleic acid formation, and energy compounds like ATP, directly influencing the photosynthesis rate and plant biomass growth (Zhao *et al.,* 2020; Xu *et al.,* 2022). The increased nitrogen availability may result from the synergistic action of mycorrhiza and carbon nanoparticles, which enhance soil physicochemical properties, microbial activity, and root nutrient uptake efficiency (Tripathi *et al.,* 2017; Bhardwaj *et al.,* 2021). Furthermore, nanomaterials can facilitate nutrient transport and stimulate enzymatic activity in the rhizosphere, enhancing plant nutrition and stress adaptation capacity (Rastogi *et al.,* 2019).

Carbon nanoparticles and mycorrhizal fungi exert multi-level effects by simultaneously promoting growth, disease resistance, and stress tolerance while improving soil quality through enhanced structure and microbial activity (Jamiołkowska *et al.,* 2020a,b; Li *et al.,* 2024; Chen *et al.,* 2023). Mycorrhizal fungi participate in shaping the soil microbiome by altering the composition and quantity of root exudates, thereby influencing the development and functioning of soil microorganisms (Jamiołkowska *et al.,* 2017, 2020a; Bücking *et al.,* 2008). Mycorrhiza can also induce qualitative and quantitative changes in root exudates in the rhizosphere soil through various mechanisms, thereby exerting an indirect influence on rhizosphere microorganisms. These changes include increased levels of sugars, amino acids, and phenolics in root exudates, which may function as chemical signals modulating soil microbiome composition (Badri and Vivanco, 2009). Thus, the mycorrhiza not only enhances nutrient availability for plants but also indirectly shapes the composition and activity of rhizosphere microbial communities, promoting beneficial bacteria while suppressing pathogens (Bücking and Kafle, 2015).

Mycorrhizal inoculation of peppers significantly modified the rhizosphere microbial composition relative to the control, showing reduced colonization of pepper roots by phytopathogenic fungi (Jamiołkowska *et al.,* 2020a). A similar relationship was observed for total bacterial counts, including *Bacillus* spp. and *Pseudomonas* spp. populations in the pepper rhizosphere. In our study, the mycorrhizal inoculation combined with carbon nanoparticles exerted the most beneficial effect on total bacterial counts, including *Bacillus* spp. and *Pseudomonas* spp. populations, while significantly reducing the overall fungal abundance in the soil community. The increase in total bacterial counts in the soil microbiome, including the genera *Bacillus* and *Pseudomonas*, has a significant impact on plant health (Kumar *et al.,* 2014). These microorganisms inhibit the growth and development of soil phytopathogens through antibiosis, competition, and parasitism. Patkowska and Konopiński (2014) demonstrated that antagonistic bacteria *Bacillus* spp. and *Pseudomonas* spp. inhibited the growth and development of *Fusarium oxysporum*, *Haematonectria haematococca*, and *Thanatephorus cucumeris* when oats were used as cover crops in field-grown scorzonera (*Pseudopodospermum hispanicum*). These microorganisms produce antibiotics that degrade cell walls and cause lysis of fungal hyphae (Krid *et al.*, 2010; Abdulkadir and Waliyu, 2012). Additionally, the effectiveness of bacteria in reducing the population of rhizospheric fungi is associated with the action of secondary metabolites, such as iron-chelating compounds (siderophores), plant defense-inducing substances (salicylic and anthranilic acids), enzymes degrading fungal cell wall components (glucanases and endochitinases), and hormonal substances (Verma *et al.,* 2012). The application of carbon nanodots (CNDs) reduced total fungal counts in the tomato rhizosphere compared to the control, though their effect was less pronounced than that of the CNDs-mycorrhiza combination. The reduction in the number of fungi and the increase in the overall bacterial count in the rhizosphere microbiome is a beneficial phenomenon, as bacteria likely suppress the development of numerous soil-borne pathogenic fungi. Patkowska and Konopiński (2014) demonstrated that soil application of carbon nanoparticles also increased total bacterial counts, including *Bacillus* and *Pseudomonas* populations. Khanna *et al.* (2021) have argued that nanoparticles exhibit both positive and negative associations with plants and rhizospheric communities. They are known to create significant challenges for soil microflora, either through direct toxicity or by altering toxin bioavailability. Moreover, their effects on microbial communities are highly dependent on soil type, pH, and organic matter content (Rajput *et al.,* 2018), highlighting the need for careful agricultural implementation.

Prolonged exposure to nanoparticles (NPs) may disrupt functional groups of soil microorganisms, thereby negatively affecting nitrogen and phosphorus cycles (Ge *et al.,* 2011). They also indirectly impair organic compound synthesis and create antagonistic relationships (Haris, 2017). NPs are colloidal, which limits their microbial uptake, and their physicochemical properties, such as size, surface charge, and coating, also play a crucial role in interactions with microorganisms (Rico *et al.,* 2011). It is hypothesized that they exert toxicity through ion dissolution and subsequent invasion through membrane disruption (Kloepfer, 2005). Primarily, free radicals generated by NPs inhibit microbial development through cell wall/nucleus damage, exopolysaccharide suppression, biofilm biosynthesis inhibition, and lipid peroxidation (Pelletier *et al.,* 2010). Moreover, nanoparticles can alter microbial gene expression, inducing changes in enzymatic activity and cellular immune responses (Zhao *et al.,* 2021).

5. CONCLUSIONS

The use of beneficial microorganisms and nanoparticles in the protection and stimulation of plant growth seems very attractive. The results of the conducted experiments indicated a positive effect of the application of mycorrhizal fungi in combination with carbon nanoparticles (Cur-CNDs, Gly-CNDs) on the stimulation of plant growth and improvement of biological parameters of the soil. The mycorrhizal fungi in combination with carbon nanoparticles (Cur-CNDs, Gly-CNDs) had a beneficial effect on the increase in the fresh and dry mass of tomato roots and the content of chlorophyll and nitrogen in tomato leaves. The combination of mycorrhiza and nanoparticles caused a more beneficial stimulation effect than the use of nanoparticles or mycorrhizal fungi alone. Mycorrhization in combination with the application of curcumin and glycine nanoparticles also improves soil biological parameters by stimulating the growth of soil bacteria, including *Bacillus* sp. and *Pseudomonas* sp. in the tomato rhizosphere. The conducted studies constitute the basis for further field work and research aimed at developing the composition of a natural preparation for plant stimulation and protection.

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