

EFFECT OF FULLERENE C₆₀ ON TOMATO PLANTS

A. Buziashvili^{1*}, S. Prylutska², A. Yemets¹

¹Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

²National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

*Corresponding author: buziashvili.an@gmail.com

Received 19 July 2024; Accepted 3 September 2024

Background. Fullerenes, one of the allotropic forms of carbon, are the focus of intensive research in nanobiotechnology. Due to their unique physicochemical properties, there is growing interest in using them to enhance plant productivity and provide protection against various stresses. However, data on the effects of fullerenes on different plants are often contradictory.

Objective. To investigate the effect of colloidal water-soluble fullerene C₆₀ on various stages of tomato development.

Methods. The study examined the influence of fullerene C₆₀ (0–50 µg/ml) on the seed germination of the Money Maker cv. and the growth and development of seedlings and adult plants. For this purpose, morphophysiological parameters such as shoot and root length, number of lateral and adventitious roots, fresh weight, accumulation of photosynthetic pigments, and nitric oxide (II) were assessed.

Results. Both positive and some phytotoxic effects of fullerene C₆₀ on tomatoes were observed. Specifically, inhibition of seedling growth and a decrease in chlorophyll *b* and carotenoid content in adult plants were noted. Positive effects included an increased seed germination rate, higher fresh weight, greater length and number of adventitious roots in tomatoes grown in the presence of fullerene C₆₀, and a higher nitric oxide (II) content in adult plants, which may contribute to enhanced stress resistance.

Conclusions. Fullerene C₆₀ at a concentration of 25 µg/ml has a predominantly positive effect on tomato development and may be considered a promising nanomaterial for plant biotechnology.

Keywords: fullerene C₆₀; seed germination; tomato development; plant biotechnology.

Introduction

Global climate changes are creating new challenges for farmers, such as reducing arable land and the increased negative impact of stress factors on crops [1, 2]. The need to overcome the negative consequences of climate change and ensure sustainable agricultural production creates an urgent demand for the development of new environmentally friendly fertilizers and plant protection agents. Uncontrolled use of inorganic fertilizers or pesticides in the long term leads to negative environmental consequences, such as disruption of the soil microbiome, water pollution, and changes in the mineral composition of the soil [3]. An alternative environmentally friendly approach to plant protection and increasing their productivity is using new achievements in nanobiotechnology, particularly nanoparticles of various natures that can positively influence plant growth and increase their resistance to different stresses. It has been shown that nanoparticles based on metals, metal oxides (Au, Ag, Ag₂O, TiO₂, ZnO, CuO, Fe₃O₄, etc.), as well as carbon nanomaterials (single- and multi-walled nanotubes, graphene, carbon quantum dots, fullerenes, etc.) have low phytotoxicity, improve

seed germination, root and shoot growth, increase photosynthesis intensity, productivity of various plant species, and their resistance to biotic and abiotic stresses [3–8]. Furthermore, due to favorable physical and chemical characteristics such as thermal stability, surface functionalization capability, hydrophobicity, antioxidant properties, and high biological compatibility, carbon nanomaterials are widely used in various fields from mechanics, optics, nanoelectronics to pharmaceuticals and medicine [9].

One of the most well-studied types of carbon nanomaterials is carbon nanotubes – cylindrical nanostructures with a diameter of 1–40 nm and a length of up to 10 micrometers, consisting of one or several layers of graphene (single-walled or multi-walled carbon nanotubes). There are many publications [3, 4, 6–9] describing the effects and mode of their action on various biological objects, from plant, animal, and bacterial cell cultures to plant or animal organisms, as well as their potential applications as carriers for drugs, genetic material, pesticides, or phytohormones, etc.

Another type of carbon nanomaterials are fullerenes – carbon nanoellipses and nanospheres composed of C_{*n*} (*n* > 20) carbon atoms connected

in penta- and hexagonal clusters forming a spherical surface. Among fullerenes, the most studied is fullerene C_{60} . The unique physicochemical properties of fullerene C_{60} such as hydrophobicity, antioxidant activity, quantum size determine its numerous biological activities, namely, the ability to penetrate cell membranes, neutralize reactive oxygen species, inhibit tumor development in animals, as well as to enhance the expression of genes responsible for synthesis of phytohormone and protection from abiotic stresses in plants, activate growth and development processes, increase the yield, photosynthesis efficiency, sugar content, and improve plant resistance to water deficit, salt stress, etc. [3–5, 8–12].

However, there is evidence of some negative effects of fullerenes on plant growth and development, as well as certain level of phytotoxicity [3–5, 7], which can be associated with the disruption of the cell walls, blockage of plant vessels by fullerene clusters [13], disarrangement of spindle microtubules and a decrease in ROS content in mitochondria, for example, after treatment with fullerene C_{70} [14]. Considering the prospects for using fullerenes in agriculture to increase crop yields and minimize the negative impact of agrochemicals on plants [15, 16], in-depth studies of the characteristics of their effects on various plant species are urgently needed.

Tomatoes are one of the most important fruit crops worldwide, including in Ukraine. As fullerenes were shown to penetrate plant cells of roots, leaves, stems, petioles and fruits [4], the penetration and intracellular localization of fullerenes into the plant depends on their physically and chemically properties and especially on their size. Water-soluble fullerene C_{60} is a non-toxic or low-toxic nanoparticles, which the authors previously proved [17]. This indicates their safe effect on the human body, so it is essential to study the effect of this type of fullerenes (C_{60}) on plants, in particular on tomatoes.

This paper presents for the first time the study of the influence of water-soluble stable fullerene C_{60} on seed germination and morpho-physiological parameters of tomato plants with the possibility of their further use in various biotechnological approaches in agriculture.

Materials and Methods

Water Solutions of Fullerene. The synthesis, analysis of its structure and stability, and preparation of an aqueous colloidal solution of fullerene C_{60} was carried out in the chemical laboratory of the Institute of Biotechnology at the Technical

University of Ilmenau (Germany) by Prof. Uwe Ritter [18]. The study used a stock solution of water-soluble fullerene C_{60} at a 150 $\mu\text{g}/\text{ml}$ concentration. Although fullerenes are hydrophobic compounds [19], in this study a novel pristine water-soluble stable fullerene was used, which was shown to increase the water conductivity of cellular membranes via incorporation in lipid bilayer and formation of channels [18], which could facilitate the water and nutrients uptake by plants.

Study of the Effects of Fullerene C_{60} on Tomato. Tomato (*Solanum lycopersicum* L.) seeds of the Money Maker cultivar were sterilized with 70% ethanol for 2 min and 5% sodium hypochlorite (NaOCl) for 15 min, rinsed three times with sterile distilled water for 10 min and then germinated on MST (Murashige and Skoog nutrient medium for Tomatoes) medium [20]. The stock solution of fullerene was applied to the surface of the solidified agar medium in Petri dishes in the appropriate volumes that corresponds with the final concentrations (25 and 50 $\mu\text{g}/\text{ml}$) of fullerene C_{60} in the MST medium. Afterward, the Petri dishes were dried under a laminar flow. Thus, the concentration of macro- and microelements in the nutrient MST medium remained the same in both the control and experimental samples. Then, tomato seeds were sown on the surface of the medium and subsequently exposed to fullerene C_{60} during cultivation and germination at 24 °C under photoperiod (16 h light/8 h dark). The frequency of tomato seed germination was evaluated 7 days after sowing. Control samples were grown under the same conditions but without the presence of fullerene C_{60} . On the 11th day, such morphometric indicators of seedlings were studied as: shoot length, main root length, average length and number of lateral and adventitious roots, and fresh weight of seedlings.

After 14 days, tomato seedlings were transferred to 15 cm long test tubes filled with vermiculite. The tubes were filled with 8 ml of Hoagland's medium [21] per tube supplemented with 25 or 50 $\mu\text{g}/\text{ml}$ fullerene C_{60} , or without fullerene C_{60} in the control samples. Every 2 days, 3–6 ml of Hoagland's medium was added to the tubes. Tomato plants were grown for 3 months, after which the effects of fullerene C_{60} on plant growth and development were assessed. Also, the effects of 25 and 50 $\mu\text{g}/\text{ml}$ fullerene C_{60} on the accumulation of photosynthetic pigments in plant leaves and nitric oxide (II) was measured.

Measurement of Photosynthetic Pigment Content. After 3 months of plant cultivation in the presence of 25 or 50 $\mu\text{g}/\text{ml}$ of fullerene C_{60} , the photosyn-

thetic pigments content in tomato leaves was determined according to the method described in [22]. For this, 100 mg of leaves were homogenized, then 1 ml of 96% ethanol and 0.1 g CaCO_3 were added to neutralize plant acids. The homogenate was transferred to 1.5 ml microcentrifuge tubes and precipitated for 5 min at 10,000 rpm at +4 °C using an Eppendorf 5417R centrifuge (Germany). The supernatant was diluted in ethanol at a ratio of 1:10 and analyzed using a Specord 200 spectrophotometer (Analytic Jena, Germany) and WinAspect Plus 4.1 software. The absorption of chlorophylls was measured at 664.4 nm and 648.6 nm, and carotenoids at 470 nm. The concentration of pigments was determined by the formulas [23]:

$$C_{\text{chl } a} = 13.36 \cdot A_{664.4} - 5.19 \cdot A_{648.6},$$

$$C_{\text{chl } b} = 27.43 \cdot A_{648.6} - 8.12 \cdot A_{662.4},$$

$$C_{\text{car}} = (1000 \cdot A_{470} - 2.13 \cdot C_{\text{chl } a} - 97.64 \cdot C_{\text{chl } b}) / 209,$$

where $C_{\text{chl } a}$ is concentration of chlorophyll *a*, $C_{\text{chl } b}$ is concentration of chlorophyll *b*, C_{car} is concentration of carotenoids, $A_{664.4}$ is absorption at 664.4 nm, $A_{648.6}$ is absorption at 648.6 nm, A_{470} is absorption at 470 nm, and the final concentration was expressed as mg per 1 g of fresh weight.

Identification of Nitric Oxide (II) Content. After 3 months of cultivation on hydroponics in the presence of 25 and 50 $\mu\text{g/ml}$ of fullerene C_{60} , the nitric oxide (II) content was measured in tomato leaves using the method described in [24]. This method is based on the colorimetric determination of nitrite anion (NO_2^-), which forms when endogenous NO in tissues contact with atmospheric oxygen. For this, 100 mg of tomato leaves were homogenized in ceramic mortars, 1 ml of distilled water was added, and the homogenate was transferred to microcentrifuge tubes. The tubes were incubated for 1 min at 98 °C and for 15 min on ice. The samples were centrifuged for 6 min at 10,000 rpm at +4 °C using an Eppendorf 5417R centrifuge, and 10 mg of modified Griess reagent (Sigma Aldrich, G4410) was added to each supernatant. The samples were thoroughly resuspended and left for 15 min at room temperature for complete reaction. The absorption was measured at 540 nm using a Specord 200 spectrophotometer (Analytic Jena, Germany) and WinAspect Plus 4.1 software. The molar concentration of nitrite anion (NO_2^-) in the samples, which corresponds to the molar concentration of NO in plant tissues, was determined using a calibration

curve previously built with standard NaNO_2 solutions. The final NO concentration was expressed as nmol per gram of fresh weight.

Statistical Analysis. All experiments were repeated three or more times. At least 50 seeds and 10 plants were used in each individual experiment. The reliability of the results was confirmed with the one-way analysis of variance (ANOVA) test. Significant differences among means were considered at *P*-value of <0.05 level. Statistical data processing was performed with the use of Microsoft Office Excel 2019 software.

Results

The Influence of Fullerene C_{60} on the Morphometric Parameters of Tomato Plants. Previously, when studying various concentrations of water soluble fullerene C_{60} using the *Alium-test* [25], it was found by us that the most effective were 25 and 50 $\mu\text{g/ml}$, so they were chosen for further study of the influence of fullerene C_{60} on tomato. First, the effect of this nanomaterial on tomato seed germination was assessed. It has been found that after 7 days, the seed germination frequency was as follows: 49.2% in the presence of 25 $\mu\text{g/ml}$ and 42.2% in the presence of 50 $\mu\text{g/ml}$ fullerene C_{60} , while in control samples the frequency of seed germination was 36.5% (Fig. 1a). Thus, data indicate that the addition of fullerene C_{60} to the nutrient medium at concentrations of 25 or 50 $\mu\text{g/ml}$ increased the germination rate of tomato seeds by 34.8% and 15.6%, respectively, compared to the control.

It was also revealed that fullerene C_{60} affects the growth and development of tomato seedlings. In particular, in 11-day-old tomato seedlings grown in the presence of it, the length of shoots and primary roots was slightly shorter than in the control. The average shoot length of seedlings growing in the presence of 25 and 50 $\mu\text{g/ml}$ of fullerene was 30.36 and 28.2 mm, respectively, while in control it was 30.52 mm. The primary root length of seedlings growing at 25 $\mu\text{g/ml}$ was 34.79 mm, 30.42 mm at 50 $\mu\text{g/ml}$ fullerene C_{60} and 38.33 mm in control (Fig. 1b, Fig. 2). Thus, in the presence of fullerene C_{60} , the shoot length of the seedlings decreased by an average of 0.53% at a concentration of 25 $\mu\text{g/ml}$ and by 7.61% at a concentration of 50 $\mu\text{g/ml}$. The length of the primary root decreased by 9.24% at a concentration of 25 $\mu\text{g/ml}$ and by 20.63% at a concentration of 50 $\mu\text{g/ml}$ compared to the control.

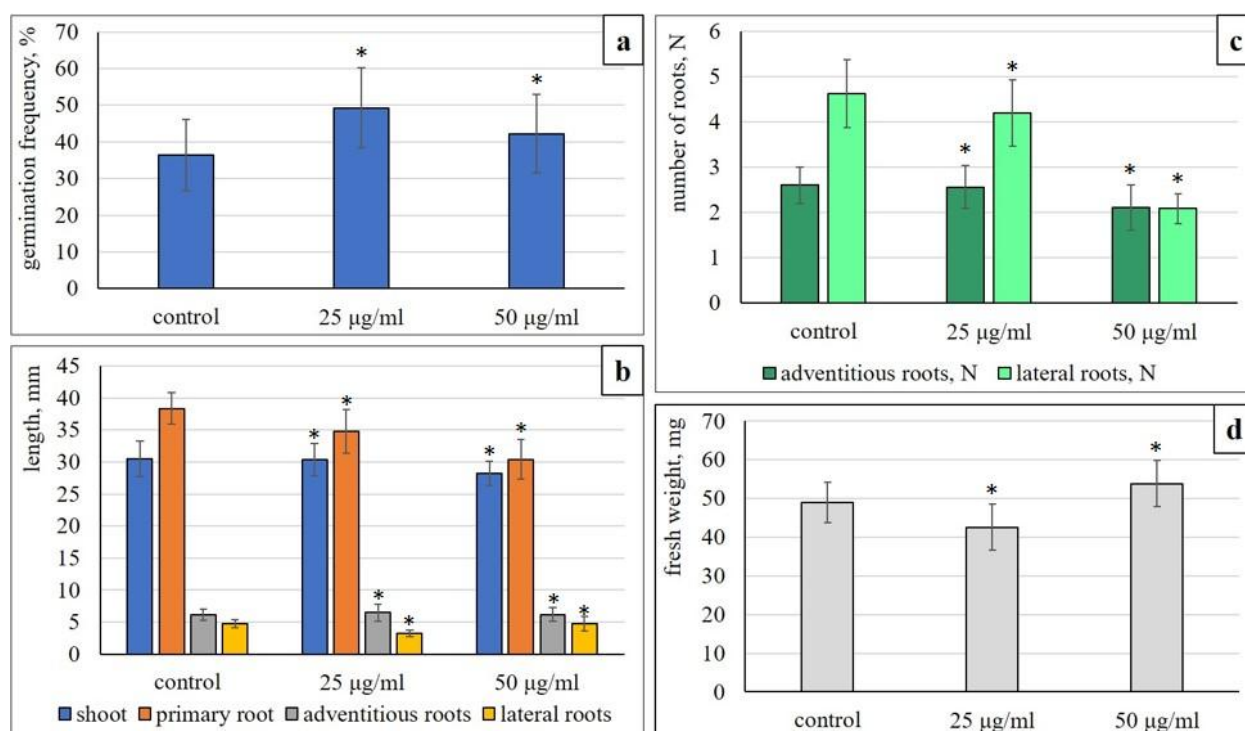


Figure 1: Seed germination frequency (a), the average length of shoots, primary, adventitious, and lateral roots (b), average number of adventitious and lateral roots (c), and average fresh weight (d) of tomato seedlings grown with the presence of 25 and 50 µg/ml fullerene C_{60} . * – $p < 0,05$ compared to control.



Figure 2: Morphology of tomato seedlings after 11 days of growing without (a) or in the presence of fullerene C_{60} at concentrations of 25 (b) and 50 µg/ml (c). Scale bar: 1 cm

Additionally, we studied the effect of fullerene C_{60} on the length and number of lateral and adventitious roots in tomatoes. Although the adventitious roots were found in all samples, both in control and grown in the presence of 25 and 50 µg/ml fullerene C_{60} , the adventitious roots due to the action of fullerene were longer than in the control. The average length of adventitious roots of 11-day-old seedlings grown at 25 µg/ml was 6.48 mm, and 6.2 mm at 50 µg/ml fullerene, whereas in the control it was approximately 6.15 mm (Fig. 1b). Therefore, in the presence of fullerene C_{60} , the adventitious roots were 5.37% and 0.8% longer than in control. In contrast, the average length of lateral roots was greater in the control seedlings (4.78 mm), while in the presence of 25 and 50 µg/ml of fullerene, it was 3.21 mm and 4.71 mm (Fig. 1b). Thus, the lateral roots were 32.84% and 1.46% shorter in the presence of fullerene compared to the control. The average number of adventitious and lateral roots was also greater in control. The number of adventitious roots was 2.6 in the control, 2.56 and 2.1 in the presence of 25 and 50 µg/ml of fullerene C_{60} , which is 1.53% and 19.23% less than in the control. The number of lateral roots was 4.63 in the control, 4.2 – at 25 µg/ml, and 2.09 – at 50 µg/ml fullerene C_{60} , respectively, (Fig. 1c), which is 9.28% and 54.86% less than in the control plants.

In addition, the average fresh weight of Money Maker tomato seedling was determined. The weight of the control seedlings was 49.04 mg, 42.61 mg – grown in the presence of 25 µg/ml, and 53.89 mg – at 50 µg/ml fullerene C₆₀. The fresh weight of seedlings grown in the presence of 25 µg/ml fullerene was 13.11% less than in control, whereas at 50 µg/ml this parameter was 9.89% greater than control one (see Fig. 1d).

In a month tomato plants grown on hydroponics supplemented with fullerene C₆₀ at both concentrations (25 and 50 µg/ml) had no significant differences in shoot and leaf morphology compared to the control (Fig. 3). However, as noted above the development of greater amounts of adventitious

roots was noted in tomato plants grown in the presence of 25 and 50 µg/ml fullerene C₆₀ than in the control plants (Fig. 4, the Table).

The influence of fullerene C₆₀ on the photosynthetic pigments content in tomato plants. It was found that the chlorophyll *a* content was at a similar level (about 3 mg/g) in control plants and plants grown in the presence of 25 and 50 µg/ml fullerene C₆₀ (Fig. 5). At the same time, the chlorophyll *b* content in the leaves of the control plants was higher than that of the plants grown in the presence of fullerene. Its level was at 3.45 mg/g of fresh weight in control plants, and in the leaves of the plants grown in the presence of 25 and 50 µg/ml fullerene, it was 1.45 and 1.5 mg/g, respectively,

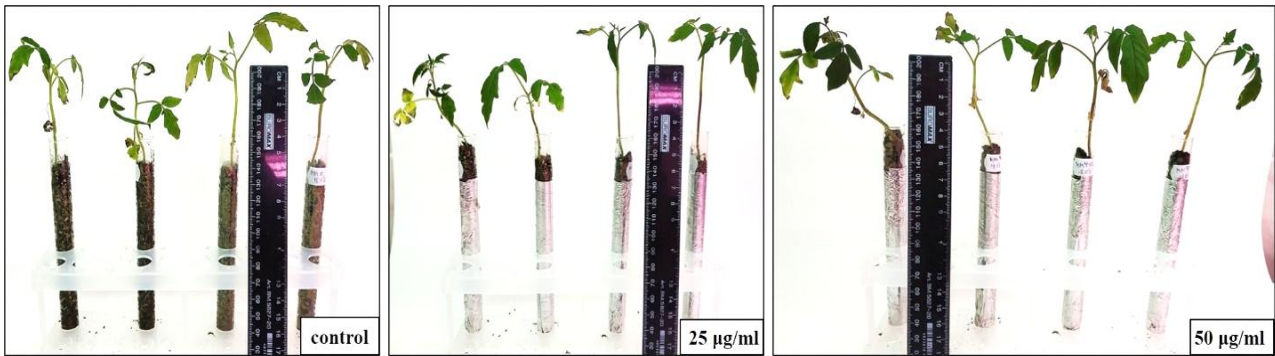


Figure 3: Tomato plants cultivated on hydroponics in the presence of fullerene C₆₀ at concentrations of 25 and 50 µg/ml

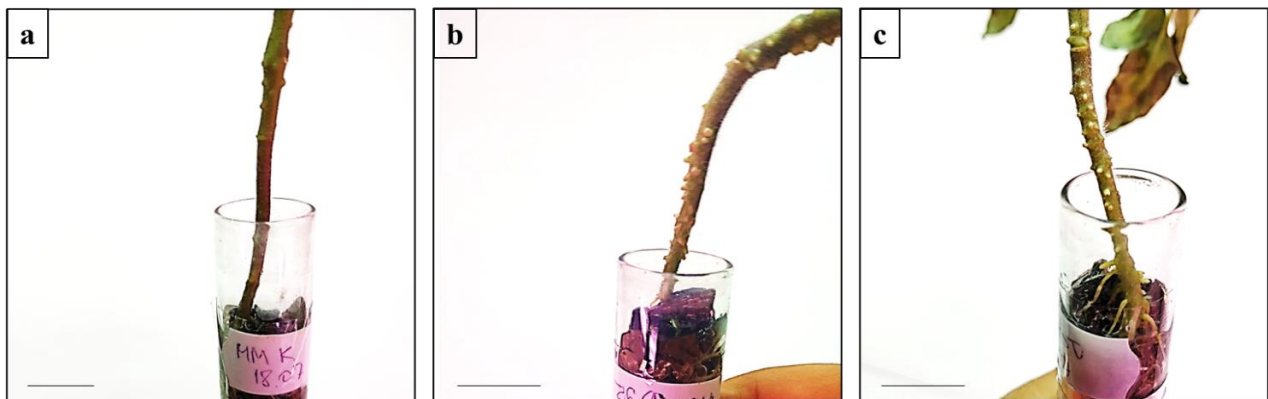


Figure 4: Adventitious roots on the stems of tomato plants after 1 month of cultivation in the presence of fullerene C₆₀: (a) control line, (b) line grown in the presence of 25 µg/ml of fullerene, (c) line grown in the presence of 50 µg/ml of fullerene. Scale bar: 1 cm

Table: Development of adventitious roots in tomato plants (experimental samples) grown in the presence of fullerene C₆₀

Fullerene C ₆₀	Sample						
	#1	#2	#3	#4	#5	#6	#7
0 µg/ml (Control)	±	+	++	+	+	+	±
25 µg/ml	+	++	++	++	± 1-2 adventitious roots, + 2-3 adventitious roots, ++ more than 3 adventitious roots		

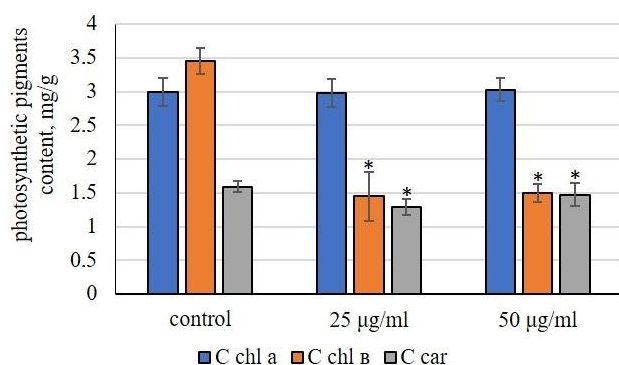


Figure 5: Photosynthetic pigments content in tomato plants grown hydroponically in the presence of fullerene C_{60} . * – $p < 0,05$ compared to control

which is 57.97% and 56.52% lower than in control. The carotenoid content in the leaves of plants grown in the presence of 25 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ of fullerene was at 1.59 and 1.29 mg/g, and 1.47 mg/g of fresh weight in the control, respectively. Thus, in the presence of fullerene C_{60} , the carotenoid content in tomato leaves was 8.16% and 12.25% lower than in the control.

The Impact of Fullerene on the Endogenous Nitric Oxide (II) Content in Tomato Plants. In this study, it was found that the NO content in the control plants, grown under hydroponic conditions for 3 months, was at a level of 11.42 nmol/g of fresh weight, while in the presence of 25 $\mu\text{g/ml}$ fullerene, the NO content reached 12.1 nmol/g, and in the presence of 50 $\mu\text{g/ml}$ fullerene – 11.77 nmol/g (Fig. 6). Thus, the nitric oxide (II) content was 5.61% and 2.97% higher in the presence of 25 and 50 $\mu\text{g/ml}$ fullerene, than in the control.

Discussion

First discovered and synthesized in the 1970s, fullerenes are widely used in nanotechnology and biomedicine nowadays. The biological effects of fullerenes and their derivatives have been the subject of intensive research over the past decade. Their functional characteristics, such as antioxidant properties, antibacterial activity, and the ability to adsorb organic pollutants and interact with a wide range of organic molecules, require comprehensive and systematic study involving various biological objects, in particular, plants [9]. When studying the effect of fullerenes on plants, it is important to evaluate, first of all, their influence on the key stages of their development, namely on seed germination, growth and development of both seed-

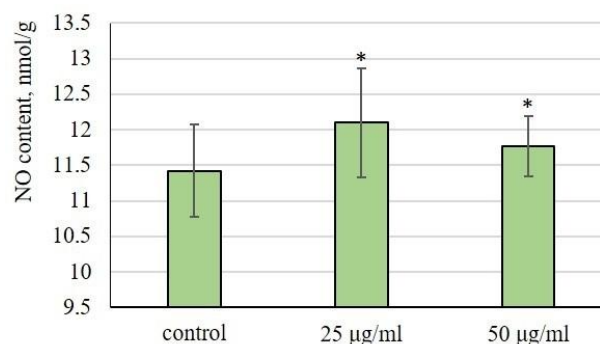


Figure 6: Nitric oxide (II) content in the leaves of tomato plants grown hydroponically in the presence of fullerene C_{60} . * – $p < 0,05$ compared to control

lings and adult plants, which was undertaken in our work to determine the peculiarities of the action of water-soluble fullerene C_{60} for tomatoes. The results of our investigation show for the first time the effects of this type of fullerene on tomato plants using the Money Maker cultivar as a well-known model plant for different physiological and biotechnological studies. In the work, both the generally used methods of analyzing the morphological parameters of tomato seedlings and plants were used, and also advanced approaches were applied, in particular, the spectrophotometric method of assessing the content of photosynthetic pigments, and the accumulation of NO in plant tissues. Taking into account that NO is an important signaling molecule involved in increasing plant resistance to various types of stress [26, 27], it was important to evaluate how fullerene C_{60} affects its endogenous level in tomatoes.

It should be noted that the results of the previous studies on the impact of fullerenes and their derivatives on plants are scarce and contradictory. Some studies report negative or neutral effects of fullerene C_{60} on the growth and development of terrestrial and aquatic plants [3, 4, 9]. For example, it was shown the inhibition of photosynthesis and magnesium uptake by phytoplankton [28], and inhibition of growth and accumulation of chlorophyll in duckweed (*Lemna gibba*) [29] under the influence of fullerenes. The other study [30] showed that the treatment of wheat (*Triticum aestivum*), rice (*Oryza sativa*), cucumber (*Cucumis sativus*), and mung bean (*Vigna radiata*) seeds with fullerene at concentrations of 10–500 mg/l, or watering of the soil with fullerene solution at a concentration of 1000 mg/kg did not affect the germination of these plants. In contrast, fullerenols,

which are OH-functionalized fullerenes, often have a positive effect on plant growth, such as stimulation of cell division of green algae *Pseudokirchneriella subcapitata* and hypocotyl growth of *Arabidopsis thaliana in vitro* [31]. Moreover, seed treatment of bitter melon (*Momordica charantia*) with fullerene even resulted in increased fruit size and yield by 128%, as well as increased content of biologically active compounds in the fruits, such as cucurbitacin-B, lycopene, charantin, and inulin [32].

In our study the positive effect of safe for human water-soluble fullerene C₆₀ on tomato seed germination was found. The most effective concentration was 25 µg/ml, as its application increased the germination rate of seeds by 12.7% compared to the control. In addition, this concentration stimulated the formation of adventitious roots and increased their length in tomato seedlings and adults plants (see the Table, Fig. 1b, Fig. 4), which is extremely important at all stages of tomato growth and development, as well as for the absorption of nutrients, water and further adaptation to stress stimuli [33–36].

Despite this positive effect of fullerene C₆₀, it was also revealed that seedlings growing in its presence were slightly retarded in growth and development (see Figs. 1 and 2), although adult plants subsequently grown in the presence of fullerene C₆₀ did not differ morphologically from control plants. Based on this, we can conclude that one of the possible mechanisms stimulating tomato seed germination and the formation of adventitious roots is mediated by fullerene [35, 36]. However, in a previous study [15] the application of fullerenes didn't cause any effects on morphometric parameters of tomato plants. This difference may be due to the use of various types of fullerenes and different tomato varieties in the experiments: in our study it was the Money Maker cv., and in [15] the Brandywine cv. was used. It should be noted that in studies [14, 30, 31], the effects of several fullerenes on various plant species (such as rice and *Arabidopsis thaliana*) were also evaluated by seed germination frequency, biomass, shoot and root lengths of seedlings. Thus, the morphophysiological indicators selected and assessed in our work are sufficient to study the effect of water soluble fullerene C₆₀ on tomatoes at the early stages of their development.

Considering that the effect of certain fullerenes on plants was studied [13, 19, 30] when they were grown under hydroponic conditions, we conducted the similar investigation with water soluble fullerene C₆₀ on tomatoes. Nowadays, hydroponic cultivation of crops is quite widespread, so it

is extremely relevant to improve the quality of crops grown under hydroponic conditions. The hydroponics conditions indeed differ from those of cultivation in open ground or greenhouse. The main difference is that hydroponics allows careful control of different abiotic stresses that affect plants. However, plants become more susceptible to phytopathogens that can form biofilms and negatively affect the quantity and quality of the yield. During the cultivation of tomato plants in hydroponics supplemented with tested concentrations of fullerene C₆₀, no significant differences in the morphology of the aboveground organs between control and experimental tomato plants were observed by us (see Fig. 4). Moreover, fullerene C₆₀ stimulated the formation of adventitious roots, which is extremely important for the development of this plant species. Thus, adding fullerene C₆₀ to a hydroponic medium can be a promising approach to increase their productivity.

Regarding biochemical stress markers – since the effect of fullerenes on different plant species is not well studied, it was unclear whether the impact of fullerenes on tomato plant growth and development would be positive or negative. Based on this, such physiological markers, as total chlorophyll content, which corresponds to photosynthesis efficiency, and endogenous NO content, a molecule that mediates many physiological processes in plants, including seed germination, growth, root development, and adaptation to abiotic stress, were chosen for evaluation of the fullerene C₆₀ effect on tomato plants [26, 27]. It was established that the effect of fullerene C₆₀ leads to an increase in the content of endogenous NO (Fig. 6) in the leaves. Although the level of endogenous NO was slightly higher (5.6% at 25 µg/ml and 2.9% at 50 µg/ml), the increased NO content in tomato plants may further enhance their resistance to various types of stress, mediated by NO [37–39]. For instance, a recent study [40] also demonstrated that fullerene increases drought tolerance in sugar beet (*Beta vulgaris*) due to its ability to bind water molecules and reduce oxidative stress caused by water deficiency. Besides its direct antioxidant activity, NO can induce stomatal closure, programmed cell death, mediate hypersensitive response and system-acquired resistance reactions during abiotic or biotic stresses [37–39].

Considering the chemical properties of fullerenes it could be supposed that the effects of fullerenes on plants might be mediated by their antioxidant properties and interaction with enzymes involved in the biosynthesis of molecules playing

an important role in the functioning of plants [7]. It is known that chlorophyll (chlorophyll *a* and chlorophyll *b*) and carotenoids play an essential role in plant photosynthesis and stress response [23]. The evaluation of chlorophyll content can be used to monitor the effects of various environmental factors, the quality of irrigation, light intensity, plant health, productivity, and more [23]. Therefore, we studied the influence of fullerene C₆₀ on the content of photosynthetic pigments (chlorophyll *a* and *b* and carotenoids) in the leaves of tomato plants. It was found that in plants grown in the presence of fullerene C₆₀, the content of chlorophyll *b* and carotenoids was significantly reduced, but fullerene C₆₀ did not affect chlorophyll *a*, its content was the same as in the control. Since chlorophyll *a* is the main pigment responsible for photosynthesis, it is important that fullerene C₆₀ did not disrupt its content in tomato tissues.

Based on the obtained results, it can be stated that, despite the insignificant negative effect on tomato seedlings, the positive effect of the studied water-soluble fullerene C₆₀ at a concentration of 25 µg/ml on the growth and development of tomatoes prevails over phytotoxicity, since it enhances seed germination, the formation of adventitious roots and the content of NO, which can be one of the physiological strategies for plant adaptation to various stresses. The use of nanobiotechnological approaches to improve plant productivity and protection can become an alternative to such biotechnological methods as cell selection, genetic engineering (including the use of relevant target genes and/or safe selectable marker genes), genome editing techniques, etc. [20, 41, 42] in nearest future.

References

- [1] Habib-Ur-Rahman M, Ahmad A, Raza A, Hasnain MU, Alharby HF, Alzahrani YM, Bamagoos AA, et al. Impact of climate change on agricultural production; Issues, challenges, and opportunities in Asia. *Front Plant Sci.* 2022;13:925548. DOI: 10.3389/fpls.2022.925548
- [2] Azeem MI, Alhafi Alotaibi B. Farmers' beliefs and concerns about climate change, and their adaptation behavior to combat climate change in Saudi Arabia. *PLoS One.* 2023 Jan 25;18(1):e0280838. DOI: 10.1371/journal.pone.0280838
- [3] Singh A, Bhati A, Gunture, Tripathi KM, Sonkar SK. Nanocarbons in agricultural plants: can be a potential nanofertilizer? In: *Nanotechnology in environmental science.* New Jersey: Wiley-VCH Verlag GmbH & Co; 2018. pp. 153-90. DOI: 10.1002/9783527808854.ch6
- [4] Husen A, Siddiqi KS. Carbon and fullerene nanomaterials in plant system. *J Nanobiotechnology.* 2014 Apr 25;12:16. DOI: 10.1186/1477-3155-12-16
- [5] Prylutska SV, Franskevych DV, Yemets AI. Cellular biological and molecular genetic effects of carbon nanomaterials in plants. *Cytol Genet.* 2022;56(4):351-60. DOI: 10.3103/S0095452722040077
- [6] Manzoor N, Ali L, Ahmed T, Noman M, Adrees M, Shahid MS, et al. Recent advancements and development in nano-enabled agriculture for improving abiotic stress tolerance in plants. *Front Plant Sci.* 2022;13:951752. DOI: 10.3389/fpls.2022.951752
- [7] Ma X, Geisler-Lee J, Deng Y, Kolmakov A. Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Sci Total Environ.* 2010 Jul 15;408(16):3053-61. DOI: 10.1016/j.scitotenv.2010.03.031

Conclusions

Summarizing the obtained data, it can be concluded that the studied stable water-soluble fullerene C₆₀ at a concentration of 25 µg/ml has a predominantly positive effect on the development of the Money Maker tomato variety. In particular, the application of water-soluble fullerene C₆₀ to tomatoes at different stages of development caused an increase in seed germination, induction of root formation, especially adventitious roots, an increase in the length of adventitious roots and an increase in the NO content in plants, which will most likely contribute to resistance to various types of biotic and abiotic stresses should they occur. Taking this into account, the new water-soluble fullerene C₆₀ can be considered as a promising nanomaterial and an alternative to modern agrochemicals, although this certainly requires a number of additional studies.

Interests disclosure

The authors declare that they have no conflict of interest.

Acknowledgements

This research was carried out in the frames of the project of the Ministry of Education and Science (2023–2025) "Regulation of intracellular mechanisms of stress resistance in agricultural plants using carbon nanomaterials" and partly under the budget program of the National Academy of Sciences of Ukraine (#0124U002424, 2024–2028).

- [8] Borovaya M, Naumenko A, Horiunova I, Plokhovska S, Blume Y, Yemets A. "Green" synthesis of Ag₂S nanoparticles, study of their properties and bioimaging applications. *Appl Nanosci*. 2020;10(12):4931-40. DOI: 10.1007/s13204-020-01365-3
- [9] Zaytseva O, Neumann G. Carbon nanomaterials: production, impact on plant development, agricultural and environmental applications. *Chem Biol Technol Agric*. 2016;3:17. DOI: 10.1186/s40538-016-0070-8
- [10] Xiong JL, Ma N. Transcriptomic and metabolomic analyses reveal that fullerol improves drought tolerance in *Brassica napus* L. *Int J Mol Sci*. 2022 Dec 4;23(23):15304. DOI: 10.3390/ijms232315304
- [11] Shafiq F, Iqbal M, Ali M, Ashraf MA. Fullerol regulates oxidative stress and tissue ionic homeostasis in spring wheat to improve net-primary productivity under salt-stress. *Ecotoxicol Environ Saf*. 2021;211:111901. DOI: 10.1016/j.ecoenv.2021.111901
- [12] Xiong JL, Li J, Wang HC, Zhang CL, Naeem MS. Fullerol improves seed germination, biomass accumulation, photosynthesis and antioxidant system in *Brassica napus* L. under water stress. *Plant Physiol Biochem*. 2018 Aug;129:130-40. DOI: 10.1016/j.plaphy.2018.05.026
- [13] He A, Jiang J, Ding J, Sheng GD. Blocking effect of fullerene nanoparticles (nC₆₀) on the plant cell structure and its phytotoxicity. *Chemosphere*. 2021 Sep;278:130474. DOI: 10.1016/j.chemosphere.2021.130474
- [14] Liu Q, Zhao Y, Wan Y, Zheng J, Zhang X, Wang C, et al. Study of the inhibitory effect of water-soluble fullerenes on plant growth at the cellular level. *ACS Nano*. 2010 Oct 26;4(10):5743-8. DOI: 10.1021/nn101430g
- [15] De La Torre-Roche R, Hawthorne J, Deng Y, Xing B, Cai W, Newman LA, et al. Multiwalled carbon nanotubes and c60 fullerenes differentially impact the accumulation of weathered pesticides in four agricultural plants. *Environ Sci Technol*. 2013;47(21):12539-47. DOI: 10.1021/es4034809.
- [16] Yemets A, Stelmakh O, Blume YB. Effects of the herbicide isopropyl-*N*-phenyl carbamate on microtubules and MTOCs in lines of *Nicotiana glauca* resistant and sensitive to its action. *Cell Biol Int*. 2008;32(6):623-9. DOI: 10.1016/j.cellbi.2008.01.012
- [17] Prylutska SV, Grebinyk AG, Lynchak OV, Byelinska IV, Cherepanov VV, Tauscher E, et al. *In vitro* and *in vivo* toxicity of pristine C60 fullerene aqueous colloid solution. *Fullerenes Nanotubes Carbon Nanostruct*. 2019;27(9):715-28. DOI: 10.1080/1536383X.2019.1634055
- [18] Schuetze C, Ritter U, Scharff P, Bychko A, Prylutska S, Rybalchenko V, et al. Interaction of N-fluorescein-5-isothiocyanate pyrrolidine-C₆₀ compound with a model bimolecular lipid membrane. *Mater Sci Engineer. C*. 2011;31(5):1148-50. DOI: 10.1016/j.msec.2011.02.026
- [19] Castro E, Garcia AH, Zavala G, Echevoyen L. Fullerenes in biology and medicine. *Mater Chem B Mater Biol Med*. 2017;5(32):6523-35. DOI: 10.1039/C7TB00855D
- [20] Buziashvili A, Cherednichenko L, Kropyvko S, Yemets A. Transgenic tomato lines expressing human lactoferrin show increased resistance to bacterial and fungal pathogens. *Biocatalysis Agricult Biotechnol*. 2020;25:101602. DOI: 10.1016/j.bcab.2020.101602
- [21] Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. 2nd edition. California Agricultural Experiment Station, Circular-347; 1950.
- [22] Tsygankova V, Andrusevich Y, Shtompel O, Kopich V, Solomyanny R, Bondarenko O, Brovarets V. Phytohormone-like effect of pyrimidine derivatives on regulation of vegetative growth of tomato. *Int J Bot Stud*. 2018;3(2):91-102.
- [23] Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol*. 1987;148C:350-82. DOI: 10.1016/0076-6879(87)48036-1
- [24] Zhang Y, Su J, Cheng D, Wang R, Mei Y, Hu H, et al. Nitric oxide contributes to methane-induced osmotic stress tolerance in mung bean. *BMC Plant Biol*. 2018 Sep 24;18(1):207. DOI: 10.1186/s12870-018-1426-y
- [25] Buziashvili AY, Melnychuk OV, Prylutska SV, Yemets AI. Investigation of phytotoxic effects of fullerene C₆₀ with the use of *Allium*-test. *Fact Exp Evol Org*. 2024;34:137-42. DOI: 10.7124/FEEO.v34.1630
- [26] Kumar D, Ohri P. Say "NO" to plant stresses: Unravelling the role of nitric oxide under abiotic and biotic stress. *Nitric Oxide*. 2023 Jan 1;130:36-57. DOI: 10.1016/j.niox.2022.11.004
- [27] Khan M, Al Azzawi TNI, Ali S, Yun BW, Mun BG. Nitric oxide, a key modulator in the alleviation of environmental stress-mediated damage in crop plants: A meta-analysis. *Plants (Basel)*. 2023 May 26;12(11):2121. DOI: 10.3390/plants12112121
- [28] Tao X, Yu Y, Fortner JD, He Y, Chen Y, Hughes JB. Effects of aqueous stable fullerene nanocrystal (nC₆₀) on *Scenedesmus obliquus*: evaluation of the sub-lethal photosynthetic responses and inhibition mechanism. *Chemosphere*. 2015;122:162-7. DOI: 10.1016/j.chemosphere.2014.11.035.
- [29] Santos SM, Dinis AM, Rodrigues DM, Peixoto F, Videira RA, Jurado AS. Studies on the toxicity of an aqueous suspension of C60 nanoparticles using a bacterium (gen. *Bacillus*) and an aquatic plant (*Lemna gibba*) as in vitro model systems. *Aquat Toxicol*. 2013 Oct 15;142-143:347-54. DOI: 10.1016/j.aquatox.2013.09.001
- [30] Kumar S, Patra AK, Datta SC, Rosin KG, Purakayastha TJ. Phytotoxicity of nanoparticles to seed germination of plants. *Int J Adv Res*. 2015;3(3):854-65.
- [31] Gao J, Wang Y, Folta KM, Krishna V, Bai W, Indeglia P, et al. Polyhydroxy fullerenes (fullerols or fullerlenols): beneficial effects on growth and lifespan in diverse biological models. *PLoS One*. 2011;6(5):e19976. DOI: 10.1371/journal.pone.0019976

- [32] Kole C, Kole P, Randunu KM, Choudhary P, Podila R, Ke PC, et al. Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). BMC Biotechnol. 2013 Apr 26;13:37. DOI: 10.1186/1472-6750-13-37.
- [33] Sharma M, Singh D, Saksena HB, Sharma M, Tiwari A, Awasthi P, et al. Understanding the intricate web of phytohormone signalling in modulating root system architecture. Int J Mol Sci. 2021 May 24;22(11):5508. DOI: 10.3390/ijms22115508
- [34] Steffens B, Rasmussen A. The physiology of adventitious roots. Plant Physiol. 2016;170(2):603-17. DOI: 10.1104/pp.15.01360
- [35] Zhang Y, Wang R, Wang X, Zhao C, Shen H, Yang L. Nitric oxide regulates seed germination by integrating multiple signalling pathways. Int J Mol Sci. 2023 May 21;24(10):9052. DOI: 10.3390/ijms24109052
- [36] Roussos PA. Adventitious Root formation in plants: The implication of hydrogen peroxide and nitric oxide. Antioxidants (Basel). 2023 Apr 2;12(4):862. DOI: 10.3390/antiox12040862
- [37] Plohovska SH, Krasnylenko YA, Yemets AI. Nitric oxide modulates actin filament organization in *Arabidopsis thaliana* primary root cells at low temperatures. Cell Biol Int. 2019 Sep;43(9):1020-30. DOI: 10.1002/cbin.10931
- [38] Plohovska SH, Shadrina RY, Kravets OA, Yemets AI, Blume YB. The role of nitric oxide in the *Arabidopsis thaliana* response to simulated microgravity and the involvement of autophagy in this process. Cytol Genet. 2022;56(3):244-52. DOI: 10.3103/S0095452722030100
- [39] Kolupaev YE, Yemets AI, Yastreb TO, Blume YB. The role of nitric oxide and hydrogen sulfide in regulation of redox homeostasis at extreme temperatures in plants. Front Plant Sci. 2023 Feb 7;14:1128439. DOI: 10.3389/fpls.2023.1128439
- [40] Borišev M, Borišev I, Župunski M, Arsenov D, Pajević S, Čurčić Ž, et al. Drought impact is alleviated in sugar beets (*Beta vulgaris* L.) by foliar application of fullerene nanoparticles. PLoS One. 2016;11(11):e0166248. DOI: 10.1371/journal.pone.0166248
- [41] Yemets A, Radchuk V, Bayer O, Bayer G, Pakhomov A, Vance Baird W, Blume YB. Development of transformation vectors based upon a modified plant alpha-tubulin gene as the selectable marker. Cell Biol Int. 2008 May;32(5):566-70. DOI: 10.1016/j.cellbi.2007.11.012
- [42] Buziashvili A, Kolomiets Y, Butsenko L, Yemets A. Biotechnological approaches for enhancing the resistance of tomato plants to phytopathogenic bacteria. Biologia Plantarum. 2023;67:305-21. DOI: 10.32615/bp.2023.034

А. Бузіашвілі¹, С. Прилуцька², А. Ємець¹

¹ДУ "Інститут харчової біотехнології та геноміки Національної академії наук України", Київ, Україна

²Національний університет біоресурсів і природокористування України, Київ, Україна

ВПЛИВ ФУЛЕРЕНУ C₆₀ НА РОСЛИНИ ТОМАТУ

Проблематика. Фулерени, одна з алотропних форм вуглецю, є предметом інтенсивних досліджень у нанобіотехнологіях. Завдяки унікальним фізико-хімічним властивостям зростає інтерес до їх використання для підвищення продуктивності рослин і захисту від різних стресів. Однак дані про вплив фулеренів на різні види рослин є дещо суперечливими.

Мета. Дослідити вплив колоїдного водорозчинного фулерену C₆₀ на різні фази розвитку томатів.

Методика реалізації. Вивчався вплив фулерену C₆₀ (0–50 мкг/мл) на схожість насіння сорту Мані Мейкер, ріст і розвиток проростків і дорослих рослин. Для цього оцінювали їхні морфологічні показники: довжину пагонів, коренів, кількість бічних, додаткових коренів, сиру масу, накопичення фотосинтетичних пігментів та оксиду азоту (II).

Результати. Встановлено як позитивну, так і певну фітотоксичну дію фулерену C₆₀ на томати. Зокрема, спостерігали пригнічення росту проростків, а також зниження вмісту хлорофілу *b* та каротиноїдів у дорослих рослин. Позитивні ефекти включали індукцію проростання насіння, збільшення сирої маси, довжини та кількості додаткових коренів томатів, вирощених за його наявності, а також підвищений вміст оксиду азоту (II) у дорослих рослинах, що, можливо, сприятиме підвищенню стійкості рослин до різних видів стресів у разі їх виникнення.

Висновки. Досліджуваний фулерен C₆₀ у концентрації 25 мкг/мл має переважно позитивний вплив на розвиток томатів і може розглядатися як перспективний наноматеріал для біотехнології рослин.

Ключові слова: фулерен C₆₀; проростання насіння; розвиток томатів; біотехнологія рослин.