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PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF WINTER WHEAT *TRITICUM AESTIVUM* L. PLANTS AFTER SEED TREATMENT WITH FULLERENE C_{60}

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Extreme climatic conditions, pests, diseases and environmental pollution significantly impact the cultivation of agricultural products and the quality of plant raw materials. It is assumed that nanostructured carbon materials, particularly fullerene C_{60}, due to antioxidant, antiviral, and antibacterial properties can be used to prevent these effects. This study aimed to evaluate the effect of pre-sowing treatment of wheat seeds with fullerene C_{60} on the state of plants 14 days after germination. The seeds of the winter wheat Triticum aestivum L. of the Patras and Akter varieties were treated with a colloidal solution of fullerene C_{60} (0.1-1.0 µg/ml) for 3 h. Biomorphometric parameters, photosynthetic pigments, phenolic compounds, MDA content and catalase activity were assessed using standard techniques. It was shown that seeds treatment with fullerene C_{60} was followed by the greater increase of both the fresh weight of Akter plants and shoot length of Patras plants as compared to untreated controls. A dose-dependent effect of fullerene C_{60} on the physiological and biochemical parameters of the plants was revealed. Photosynthetic activity in plants of both wheat varieties was enhanced after seed treatment with C_{60} in low (0.1-0.2 µg/ml) concentrations as evidenced by the increased content of chlorophylls a, while at high (0.5-1.0 µg/ml) C_{60} concentrations it decreased against the background of increased carotenoids content. The enhancement of antioxidant defense induced by C_{60} treatment at concentrations of 0.5-1.0 µg/ml was observed, as indicated by an increase in the content of phenolic compounds and activation of catalase. The positive effect of wheat seeds treatment with fullerene C_{60} indicates the potential use of carbon nanoparticles in agrobiotechnologies to improve plant growth and stress resistance.

Keywords: wheat germination, seed treatment, fullerene C_{60}, chlorophylls, carotenoids, MDA, catalase, phenolic compounds.

Food security in Ukraine and globally has become a pressing issue in recent years. Military conflicts in Europe, environmental pollution, extreme climate changes, pests, and diseases pose significant threats to agricultural production. Consequently, the exploration of new methods and the development of innovative biotechnological approaches in agriculture are essential for addressing this complex food challenge. The rapid progress of nanotechnology has led to the widespread application of nanomaterials in various industries, including materials science, energy, environmental remediation, pharmaceuticals and medicine, and agriculture. Research into the interactions between various types and structures of nanoparticles with plants and their use in agriculture has attracted much interest [1-3].

Carbon nanoparticles (CNPs), including fullerene C_{60}, graphene, graphene oxide, and both single-walled and multi-walled carbon nanotubes, are promising in this context. These materials are distinguished by their nanoscale dimensions, chemical stability, hydrophobic properties, and a wide range of biological activities, which enhance their applicability in the agro-industrial sector [4-6].

CNPs are capable of penetrating through the cell walls and membranes of plants, which constitutes their biological action. For example, the accumulation of fullerene and its water-soluble derivatives has been detected in the tissues of vascular vessels, surrounding cells, and intercellular spaces of the rice (*O. sativa*) [7], the root system of soybeans (*G. max*), tomatoes (*S. lycopersicum*), as well as in the roots and shoots of zucchini (*C. pepo)* [8], in radish (*Raphanus sativus*) [9]. There is evidence that CNPs accumulate in plant cells' subcellular organelles, particularly in plastids, vacuoles, and the nucleus [10, 11]. Single-walled carbon nanotubes (SWCNTs) penetrated the chloroplast membrane by passive diffusion and affected photosynthetic activity by transferring electrons into the photosynthetic electron transport chain [12, 13]. The penetration and accumulation of CNPs in plant cells depend on their physical and chemical properties, including molecular size, concentration, modification, surface charge, presence of functional groups, treatment conditions, and specific plant species.

CNPs can accumulate in plant tissues and contribute to the synthesis of carbon-containing bioorganic compounds. It was shown that carbon nanomaterials induced the synthesis of biologically active substances and metabolites such as phenolic compounds, carotenoids, vitamins, and glutathione in plant cells [14-17].

CNPs can alter protein activity and induce ROS formation. At low concentrations (10–250 mg/l), they positively regulate the activity of genes responsible for plant integrity, including those involved in the glutathione-ascorbate cycle, the shikimic acid pathway, and the phenylpropanoid pathway [18-20]. CNPs in plants induce the activity of antioxidant enzymes and the synthesis of secondary metabolites [21].

Carbon nanomaterials are effective as pesticides [22], seeds and plants growth enhancers [23, 24], transporters of biological active molecules to plant tissues or cells [25-27].

However, there are also literature data on the phytotoxic properties of CNPs. Thus, at high concentrations, CNPs exhibited a toxic effect on the protoplasts of common gooseberry (*Arabidopsis thaliana* – dicotyledons) and Japanese rice (*Oryza sativa subsp. japonica* – monocots) [28]. The phytotoxic effect of CNPs was accompanied by excessive production of ROS and the development of oxidative stress [28-30].

In addition, nanoparticles of fullerene (nC_{60}) reduced transpiration processes at the cellular level in plants, blocked root pores, compressed the structure of root endothelial cells, and altered the degree of saturation of fatty acids in the root cell membrane [31].

Information on the phytotoxic effect of CNPs, their impact on plant cells and organisms, mechanisms of interaction, and effects of CNPs in agro-systems is scarce and contradictory. Therefore, the study aimed to investigate the effect of fullerene C_{60} on morphological, physiological and biochemical parameters of wheat and to determine the range of non-toxic concentrations.

Materials and Methods

Preparation of water-soluble carbon nanoparticles. Aqueous colloidal solutions of fullerene C_{60} at an initial concentration of 0.15 mg/ml were obtained by evaporation of C_{60} molecules from the organic solvent toluene and transfer to the aqueous phase by sonication [32]. The structural organization of fullerene C_{60} nanoparticles and their high stability in aqueous solution were confirmed through high-resolution microscopy and dynamic light scattering [33]. The study utilized aqueous colloidal solutions of C_{60} fullerene in the following concentration range: 0.1, 0.2, 0.5, and 1 μg/ml.

Experimental conditions. Certified seeds of winter wheat *Triticum aestivum* L., varieties Patras and Akter, were used in the studies [34]. Wheat seeds were planted in a universal soil mixture produced by *Floriada* with the following composition: turf ground, high-moor peat, lowland peat, river sand, mineral fertilizers (N, P, K), trace elements (Fe, Zn, B, Cu, Mo, Mn), pH 5.5-7.5 [35]. Pre-sowing treatment is more effective because it minimizes environmental impact and enhances germination and root formation in plants. Pre-sowing treatment of wheat seeds (Fig. 1) included the following steps: 1) sterilization using 3% H_2O_2 for 7 min, 2) rinsing with distilled water three times, 3) soaking in an H_3BO_3 solution (0.6 g/l) for 20 min, 4) treatment (20 seeds per experimental group) with an aqueous colloidal solution of C_{60} fullerene at the respective concentrations for 3 h (experimental group No. 1 – 0.1 μg/ml C_{60}, experimental group No. 2 – 0.2 μg/ml C_{60}, experimental group No. 3 – 0.5 μg/ml C_{60}, experimental group No. 4 – 1.0 μg/ml C_{60}). Wheat seeds in the control group were soaked in distilled water.

Before planting in the soil mixture, the seeds were placed in Petri dishes with moist filter paper and allowed to germinate in the dark at a temperature of 17°C for two days. Following germination, the seeds were transferred to a light environment, planted in moist soil, and grown at 20°C with a 15/9 photoperiod.

Morphological and biochemical parameters in control plants (without CNP treatment) and experi-

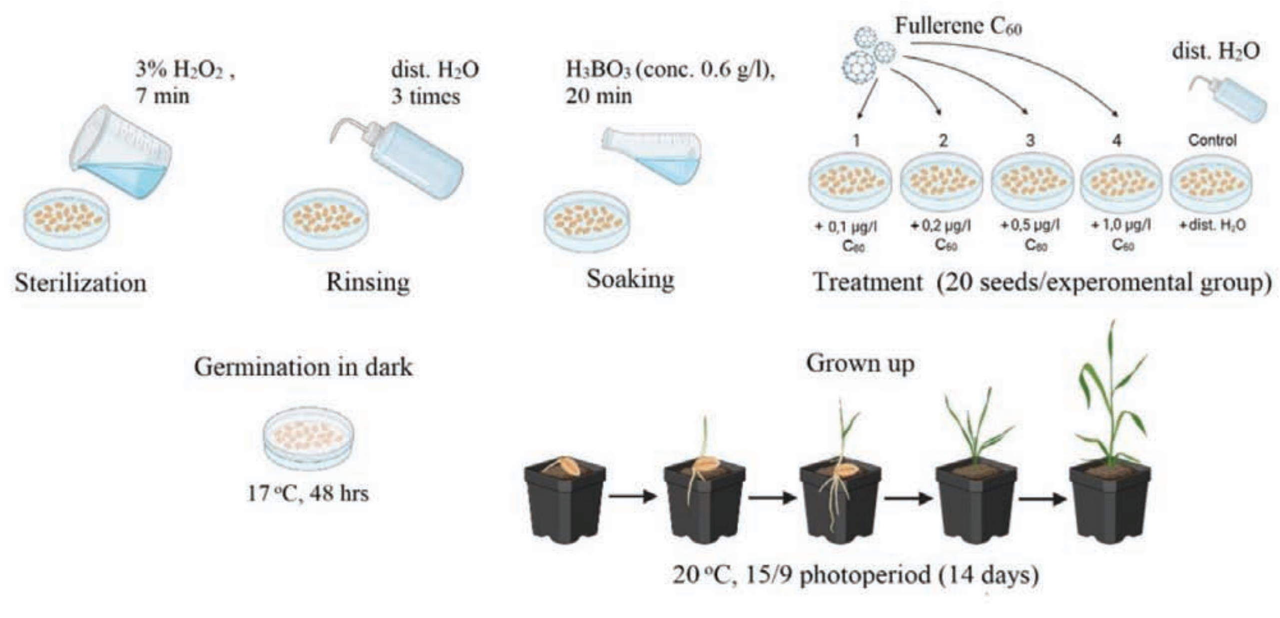

Fig. 1. Pre-sowing treatment and growing up scheme of Triticum aestivum L. wheat seeds

mental plants (CNP-treated) were evaluated on the 14th day after seed germination. The selection and preparation of plant material, along with the assessment of morphometric parameters (shoot and root length, plant weight), were conducted using standard techniques.

The content of photosynthetic pigments was determined by the spectrophotometric method. A total of 0.1 g of plant material was homogenized in a porcelain mortar with a small amount of $CaCO_3$ and then extracted with 96% C_2H_5OH, and ethanol was added to the discolored filtrate to a total sample volume of 10 ml. The optical absorption of the alcohol extracts from the experimental samples was measured at wavelengths of 665 nm for chlorophyll *a*, 649 nm for chlorophyll b, and 441 nm for carotenoids using a UVmini-1240 SHIMADZU spectrophotometer (Japan) [36]. The concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoids were determined using standard formulas:

$$C_{chl\ a} = 13.95 \times \lambda_{mean}\ 665 - 5.76 \times \lambda_{mean}\ 649,$$
$$C_{chl\ b} = 25.8 \times \lambda_{mean}\ 649 - 7.6 \times \lambda_{mean}\ 665,$$
$$C_{car} = 4.695 \times \lambda_{mean}\ 441 - 0.268 \times (C_{chl\ a} + C_{chl\ b})$$

Additionally, the total chlorophyll content, the ratio of chlorophyll *a* to chlorophyll *b*, and the ratio of total chlorophyll to carotenoids were calculated.

The content of malondialdehyde (MDA) was determined by its reaction with thiobarbituric acid (TBA) in an acidic environment at high temperature, resulting in the formation of a colored complex with an absorption maximum at a wavelength of 532 nm [37, 38]. To precipitate the protein, 0.5 g of plant material was thoroughly ground in 3 ml of 5% trichloroacetic acid in a porcelain mortar and centrifuged at 5000 g for 15 min. A 0.8% aqueous solution of TBA was added to the supernatant, and the samples were incubated in a water bath for 10 min at 100°C. The optical density was measured at 532 nm using a UVmini-1240 SHIMADZU spectrophotometer (Japan). The content of thiobarbituric acid reactive substances (TBARS) was calculated using the molecular extinction coefficient (1.56×10^5 $cm^{-1} \cdot M^{-1}$).

The total content of phenolic compounds in plant samples was determined by a modified Folin-Ciocalteu method spectrophotometrically at optical absorption at 765 nm [39]. 25-50 mg of plant material was homogenized and extracted in 1 ml of 96% ethanol at 4°C for 24 h. After extraction, ethanol in a ratio of 1:10 was added to the supernatant and mixed thoroughly. To 0.2 ml of this mixture, 2 ml of the working solution of Folin-Ciocalteu reagent and 2 ml of a 7.5% Na_2CO_3 solution were added. The tubes were then incubated at room temperature for 1 h. The optical density was measured at 765 nm using a UVmini-1240 SHIMADZU spectrophotometer (Japan). The total content of phenolic compounds was expressed in mg per 1 g of the plant material.

The activity of catalase (EC 1.11.1.6) was assessed using a spectrophotometric method that relies on the reaction between hydrogen peroxide and ammonium molybdate. This reaction produces a stable yellow-colored complex, and the intensity of its absorption was measured at a wavelength of 410 nm [40]. 0.1 g of plant material was homogenized in a porcelain mortar with a cooled 1 M phosphate buffer (pH 7.8). The resulting homogenate was centrifuged at 3,000 rpm/min for 10 min. To 50 µl of the supernatant, 1 ml of 0.03% H_2O_2 was added and incubated in a water bath at 37° C for 10 min. The reaction was stopped by adding 1 ml of ammonium molybdate, and the absorbance of the samples was measured at a wavelength of 410 nm using a UVmini-1240 SHIMADZU spectrophotometer (Japan). The enzyme activity was calculated using an H_2O_2 extinction coefficient of 22.2×10^3 $mM^{-1}\cdot cm^{-1}$.

Statistical analysis of the obtained results was performed using variance analysis ANOVA, software Microsoft Excel 2010, and GraphPad Prism 7. The results were considered reliable at *P*-values < 0.05.

Results and Discussion

Wheat is the primary grain crop in Ukraine and globally. An important condition for its cultivation is the selection of varieties that combine high yields with resistance to biotic and abiotic stressors. Wheat varieties Patras and Akter are characterized by high yields and flour quality. Additionally, the Patras variety of wheat has comprehensive winter hardiness and resistance to snow mold, while the Akter variety is resistant to lodging (8.8 points), shattering (8.7 points), root rot (7 points), septoria (9 points), fusarium (8 points), brown rust (6 points) and powdery mildew (8 points). These varieties are intensively cultivated in Ukraine and Germany [34, 41, 42].

Various environmental factors resulting from climate change and combat actions on the territory of Ukraine are contributing to a decline in the productivity of crops and the quality of plant raw materials. Consequently, the production of agricultural products necessitates the adoption of new, highly efficient biotechnological approaches that have minimal environmental impact. In this context, nanoparticles of various types are emerging as promising and effective tools in modern agricultural technologies [43]. Nanostructured carbon materials, owing to their nanoscale dimensions, hydrophobicity, antioxidant, antiviral, and antibacterial properties, can be used to regulate nutrition, increase yields, and enhance the stress resistance of agricultural plants to biotic and abiotic environmental factors.

Exogenous compounds, in particular nanoparticles, are known to inhibit the functional activity of plants and affect their growth and development. Therefore, we initially investigated the influence of carbon nanoparticles on the growth of the aboveground parts and roots, as well as the accumulation of mass in *T. aestivum* plants. For this purpose, 14 days after wheat germination, a morphometric analysis of seedlings from two varieties, Akter and Patras, treated with a solution of fullerene C_{60} at the studied concentration range of 0.1, 0.2, 0.5, and 1 µg/ml, was conducted. The average values of shoot and root lengths, as well as fresh weight, were evaluated for each sample (Table 1).

No significant differences in morphometric parameters were found between Patras and Akter varieties on the 14th day after germination of untreated (control) wheat seeds (Table 1). After treatment of seeds with fullerene C_{60}, no changes in the length of shoots and roots of the 14-day-old plants of *T. aestivum* of Patras variety were detected, while their fresh mass increased by 1.3, 1.5, 1.4 and 1.2 times at concentrations of 0.1, 0.2, 0.5 and 1 µg/ml, respectively, compared to the control (Table 1). In the Akter variety of wheat, the shoot length increased by 1.3 times under the influence of fullerene C_{60} at concentrations of 0.2, 0.5 and 1 µg/ml, while the functional activity of the roots and the fresh mass of the plants remained unchanged, with the indicators at the level of control values. Thus, carbon nanoparticles did not cause phytotoxic effects on *T. aestivum* plants and exhibited a stimulating effect on the growth of aboveground biomass.

Phytotoxicity is an important indicator for determining the potential impact of nanoparticles on the environment and plants. Numerous experimental data have shown that carbon nanoparticles exhibit toxic effects on plants, which depend on the nature, modification, bioavailability, and concentration of the molecules, treatment conditions, duration of exposure, species, and other factors [14, 44]. Authors [45] studied the dose-dependent effects of multi-walled carbon nanotubes (MWNTs) at concentrations of 125, 250, 500, and 1000 mg/l on red spinach (*Amaranthus tricolor* L.). After 15 days in hydroponic culture, plant growth, root lengths, number and size of leaf significantly decreased following exposure to 1000 mg/l MWNTs, which was also accompanied by cell death due to ROS production.

Table 1. Morphometric indicators of 14-day-old plants of T. aestivum (M ± m, n = 20)

Option experiment	Shoot length, mm	Root length, mm	Fresh weight (FW), g
		Patras variety	
Control	32.16 ± 2.66	54.36 ± 2.66	0.18 ± 0.02
+ C_{60} 0.1 μg/ml	29.46 ± 3.96	61.06 ± 3.96	0.24 ± 0.08*
+ C_{60} 0.2 μg/ml	27.97 ± 1.94	61.55 ± 1.94	0.27 ± 0.05*
+ C_{60} 0.5 μg/ml	33.47 ± 2.53	60.67 ± 2.53	0.25 ± 0.02*
+ C_{60} 1 μg/ml	33.00 ± 1.28	64.14 ± 1.28	0.22 ± 0.05*
		Akter variety	
Control	30.36 ± 3.93	57.96 ± 4.23	0.22 ± 0.01
+ C_{60} 0.1 μg/ml	31.66 ± 5.53	57.38 ± 4.63	0.23 ± 0.10
+ C_{60} 0.2 μg/ml	38.75 ± 2.81*	59.70 ± 4.43	0.25 ± 0.03
+ C_{60} 0.5 μg/ml	38.87 ± 3.42*	62.26 ± 4.02	0.25 ± 0.06
+ C_{60} 1 μg/ml	38.80 ± 2.29*	66.22 ± 3.43	0.24 ± 0.05

Note. *$P < 0.05$ in comparison with control (untreated plants)

The absence of negative effects of carbon nanotubes (CNTs) on plants is supported by the following literature data. For instance, CNTs *in vitro* and *in vivo* experiments stimulated cell growth, germination, and callus growth in tobacco and tomatoes [46, 47]. After 50 days of tobacco incubation in culture, in the presence of MWCNTs and graphene at a concentration of 50 μg/ml, the dry biomass of the callus significantly increased by 33% compared to the control. The germination rates of tomato seeds (on day 5) and the average root length of tomato seedlings (on day 20) after the application of 50 and 100 μg/ml MWCNTs or graphene were significantly higher compared to the control seedlings.

Fullerene C_{60} and graphene oxide at a concentration of 25 μg/ml increased the germination rate and viability of tomato seeds of the Money Maker variety, induced root formation, and enhanced the length of adventitious roots [48].

Nonspecific reactions in plants to stress factors, including exogenous compounds, can occur at the level of membranes, cells, and organelles. Protective adaptation and resistance to adverse environmental factors allow plants to maintain their morphological structure and functional activity. An important role in the adaptation to stress factors in photosynthetic plants is played by the pigment system, which is sensitive to exogenous compounds [49].

Nanoparticles, depending on their size, nature, solubility, concentrations, and properties, are capable of penetrating plant cells and accumulating in chloroplasts [50]. Heavy metals were shown to cause the destruction of chloroplasts, accompanied by an increase in the activity of chlorophyllase, an enzyme that degrades the chlorophyll-protein-lipid complex of the photosynthetic apparatus, and a decrease in the intensity of photosynthesis [51, 52], whereas carbon nanoparticles are capable of penetrating through plant cell walls and membranes, interacting and exchanging electrons with photosynthetic complexes *in vitro* [13].

Plant biomass depends on the utilization of absorbed light in photosynthetic processes. Thus, increasing the efficiency of light utilization by photosystem II (PSII) reaction centers in photochemical reactions enhances the overall biomass of plants [53], whereas a reduction in photosynthetic reactions leads to a decrease in wheat biomass [54]. The pigment system ensures both biomass growth and the processes of energy and plastic metabolism. Therefore, the content of pigments, their composition, and ratio in the photosynthetic organs of plants are important indicators that characterize the functional state of plants.

It was found that the total chlorophyll content in wheat is variety-specific. For instance, in 14-day-old wheat plants of the Patras variety, the content of chlorophyll *a* and carotenoids was 1.3 and 1.6 times higher, respectively, compared to the Akter variety (Fig. 2). The obtained results indicate the winter hardiness of the Patras variety, as after the resumption of vegetation in spring, the plants exhibit a bright, saturated green color of leaves and stems, which indicates active absorption of nutrients and moisture during the early stages of vegetation.

Additionally, the pigment system serves as a marker for the influence of exogenous compounds. Therefore, the next task was to study the effect of fullerene C_{60} at concentrations ranging from 0.1 to 1.0 µg/ml on the photosynthetic activity of wheat plants. A dose-dependent effect of C_{60} fullerene on the content of the studied pigments was observed. Specifically, in the Patras wheat variety, a significant increase in chlorophyll content was noted at low concentrations of 0.1 and 0.2 µg/ml of C_{60} fullerene, with chlorophyll a increasing by 43 and 40%, and chlorophyll *b* by 72 and 56%, respectively (Fig. 2). In contrast, after exposure to C_{60} fullerene at a concentration of 1 µg/ml, the content of chlorophyll *a* and chlorophyll *b* decreased by 26 and 20%, respectively, compared to plants not treated with nanoparticles. A concentration of 0.5 µg/ml of C_{60} did not affect the chlorophyll content in the Patras wheat variety.

In the Akter wheat variety, a similar effect of nanoparticles on pigment content was observed. Specifically, in plants treated with 0.1 µg/ml of C_{60}fullerene, the content of chlorophyll a increased by 28%, while at concentrations of 0.5 and 1 µg/ml, the content of chlorophylls a and b decreased by 22 and 25, and 18 and 29%, respectively, compared to the control (Fig. 2). A concentration of 0.2 µg/ml of C_{60} had no effect on the chlorophyll content in the Akter wheat variety.

The total chlorophyll content characterizes photosynthetic activity, based on the sum of the concentrations of chlorophylls *a* and *b*, which are involved in the absorption of light energy. The total chlorophyll content in both wheat varieties slightly increased at low concentrations and decreased at high concentrations of C_{60} fullerene (Table 2) compared to the control.

The ratio of chlorophylls *a*/*b*, or the chlorophyll index, allows for the assessment of the efficiency of solar energy utilization for the physiological needs of plants, making it an indicator of photosynthetic activity as well. It can also be used as a marker of resilience under stress conditions [55, 56]. The obtained results indicate sufficient resilience of the green pigments in winter wheat (*Triticum aestivum* L.) to the effects of C_{60} fullerene nanoparticles, as the chlorophyll *a*/*b* ratio values corresponded to the control values, and in some cases, were even higher compared to the control (Table 2).

Some researchers [13] have studied the concentration-dependent effect of SWCNTs loaded with polyethylene polymer on the structural and functional characteristics of pea plants (*Pisum sativum*). Under foliar application conditions, by spraying 14-day-

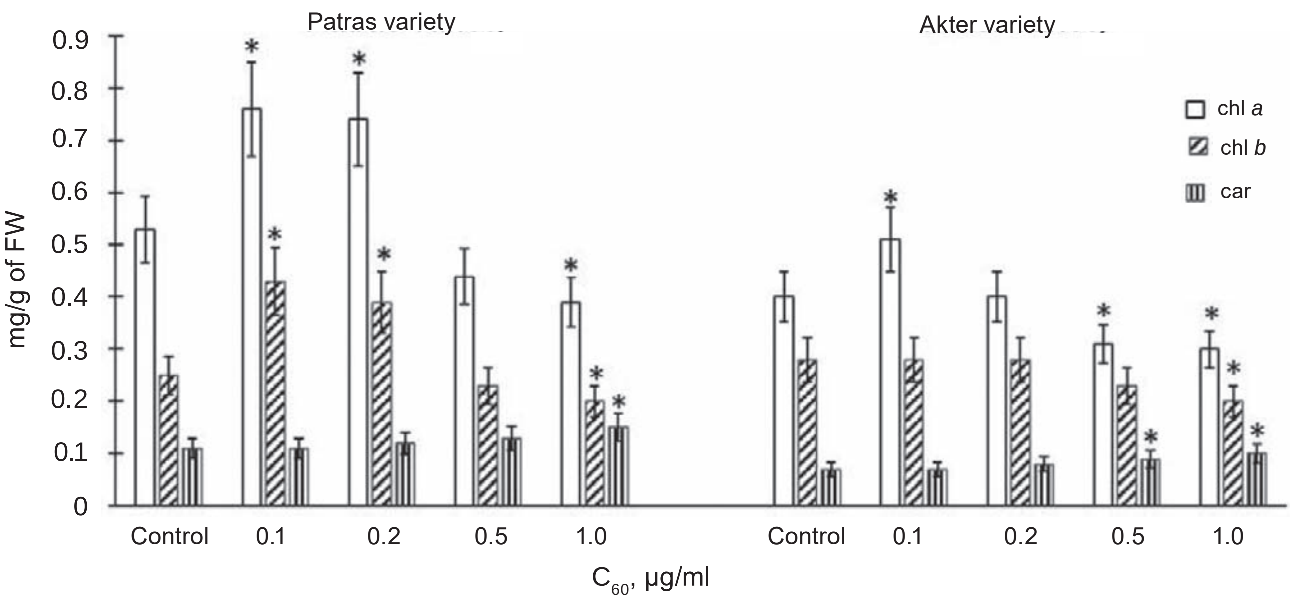

*Fig. 2. Photosynthetic pigments content (mg/g of fresh weight (FW)) in 14-day-old wheat plants treated with fullerene C_{60}. *P < 0.05 in comparison with control (untreated plants)*

Table 2. The ratio of photosynthetic pigments in 14-day-old T. aestivum sprouts treated with C_{60} fullerene

Experiment conditions	Pigments ratio		
	∑ Chl (*a* + *b*), mg/g (FW)	Chl *a*/Chl *b*	∑ Chl/∑ Car
Patras variety			
Control	0.78 ± 0.06	2.12 ± 0.19	6.88 ± 0.66
$+C_{60}$ 0.1 µg/ml	1.19 ± 0.08	1.78 ± 0.16	10.82 ± 0.96*
$+C_{60}$ 0.2 µg/ml	1.13 ± 0.09	1.90 ± 0.18	9.42 ± 0.86
$+C_{60}$ 0. 5 µg/ml	0.67 ± 0.06*	1.89 ± 0.18	5.15 ± 0.46*
$+C_{60}$ 1 µg/ml	0.59 ± 0.06*	1.91 ± 0.19	3.90 ± 0.26*
Akter variety			
Control	0.68 ± 0.07	1.43 ± 0.13	9.71 ± 0.88
$+C_{60}$ 0.1 µg/ml	0.79 ± 0.08	1.82 ± 0.17	11.29 ± 0.12
$+C_{60}$ 0.2 µg/ml	0.68 ± 0.07	1.43 ± 0.13	8.50 ± 0.76
$+C_{60}$ 0.5 µg/ml	0.54 ± 0.06*	1.35 ± 0.12	6.00 ± 0.56*
$+C_{60}$ 1 µg/ml	0.50 ± 0.06*	1.50 ± 0.14	5.00 ± 0.46*

Note. *$P < 0.05$ in comparison with control

old pea plants with SWCNTs at a concentration of 300 mg/l, a significant accumulation of epicuticular wax was observed after 7 days, along with swelling of the granal and stromal regions of thylakoid membranes and modification of chloroplast ultrastructure. The obtained results indicated a negative impact of SWCNTs on photosynthesis and a slowdown in photoprotection mechanisms, although the function of photosystem II was preserved. In contrast, SWCNTs in the concentration range of 10-100 mg/l did not cause negative effects on the studied characteristics of pea leaves.

In the functioning of the photosynthetic apparatus, carotenoids play an important role, characterized by their photoprotective and antioxidant properties [57, 58]. Thus, the content of carotenoids increased in wheat plants of both varieties at a C_{60} fullerene concentration of 1.0 µg/ml by 36 and 43%, respectively, compared to the control. Carotenoids are capable of protecting chlorophylls from photooxidation, and an increase in carotenoid content indicates their protective properties for the photosynthetic system, as carotenoids are precursors of antioxidant compounds. Conversely, a reduction in their protective properties leads to a decrease in chlorophyll content and suppression of plant growth processes [57-60].

We have shown that under the influence of C_{60} molecules, the content of carotenoids and chlorophylls increased, indicating a modification in the functioning of the light-harvesting complex of pigment systems and the activation of protective mechanisms to prevent the loss of functional capacity of thylakoid membranes. On the one hand, the activation of pigment photosystems prevents photodamage to the photosynthetic apparatus, but on the other hand, it leads to a reduction in the proportion of solar energy utilized in photosynthetic processes [61]. In addition, photoinhibition and recovery from it may be associated with significant losses in photosynthetic efficiency [62].

The ratio of total chlorophyll to carotenoids indicates how optimal the growing conditions for plants are. The ratio between the total content of chlorophylls and carotenoids decreased with increasing C_{60} concentration compared to the control (Table 2).

Thus, the increase in the studied indicators of photosynthetic activity in wheat plants under the influence of fullerene C_{60} shows a high potential intensity of photosynthesis and confirms the complex nature of the formation of protective reactions.

We observed a decrease in the content of chlorophylls *a* and *b* and total chlorophylls against the background of increased carotenoids in both wheat varieties treated with fullerene C_{60} at high concentrations (0.5 and 1 µg/ml), which indicates a decrease in photosynthetic activity and physiological processes.

Heavy metals are known to be hazardous pollutants for the environment and humans, and also to have a negative effect on the photosynthetic system of plants. For example, lead caused structural changes in the photosynthetic apparatus, induced a decrease in biosynthesis and chlorophyll degradation, and increased carotenoid synthesis in the leaves of wheat (*Triticum durum* and *T. aestivum*), barley (*Hordeum vulgare*) and oats (*Avena sativa*) [63].

Stress caused by carbon nanoparticles at high concentrations in plants was accompanied by changes in the photosynthetic system, but the mechanism is not well understood. It is assumed that due to the increased activity of photosynthetic reactions during electron transfer in photosystems I and II, reactive oxygen species can be formed, especially under intense light conditions [62]. Carotenoids absorb excess light energy and neutralize free radicals, thereby protecting plant cells from oxidative stress [57, 59].

Oxidative stress, which occurs as a result of excessive formation of reactive oxygen species (ROS) that damage the structural components of membranes and cells, is one of the causes of chlorophyll loss under stress. A marker of oxidative stress at the level of plasma membranes is MDA (malondialdehyde), which is formed during lipid peroxidation (LPO) caused by the excessive formation and accumulation of ROS [64].

We have demonstrated that the presence of fullerene C_{60} intensifies lipid peroxidation (LPO) processes in wheat, exhibiting a dose-dependent relationship (Fig. 3). Specifically, the concentration of thiobarbituric acid reactive substances (TBARS) in 14-day-old wheat seedlings of both varieties, Patras and Akter, increased with higher concentrations of nanoparticles, particularly showing a 1.7-fold increase at a concentration of 1 μg/ml of C_{60} fullerene.

An increase in the level of TBARS after exposure to C_{60} fullerene at concentrations of 0.2 μg/ml and above indicates a negative effect of nanoparticles on cell membranes due to the activation of LPO processes and the development of oxidative stress.

It has been shown that graphene oxide nanoparticles in the concentration range of 5-150 mg/l did not affect the MDA content in rapeseed (*Brassica napus* L.), rice (*Oryza sativa* L.) [65, 66], while at high concentrations of 200-250 mg/l, the MDA level increased significantly in rice plants [67].

To prevent excessive synthesis of ROS, neutralize their damaging effects, and maintain intracellular homeostasis, the cell has an antioxidant defense system, which is represented by several antioxidant enzymes. In particular, catalase (EC 1.11.1.6) is one of the main antioxidant enzymes that plays an important role in the mechanisms of antioxidant defense of plants, provides detoxification of hydrogen peroxide, reducing its toxic effect in cells [68].

In plant cells, catalase is localized in the peroxisomes and cytosol and plays an important role in photosynthetic processes, in particular in photorespiration [40]. Catalase prevents the toxic effects of

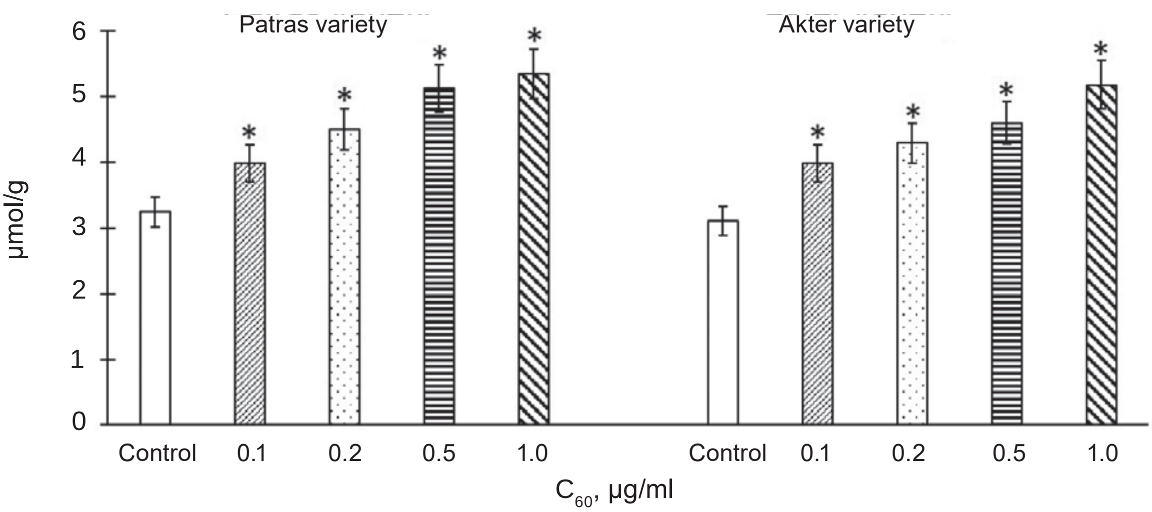

*Fig. 3. TBARS content (μmol/g) in wheat plants treated with fullerene C_{60}. *P < 0.05 in comparison with control (untreated plants)*

oxidative stress and protects chloroplasts from oxidative damage, thereby ensuring the stable operation of photosystems I and II.

We observed the activation of catalase in wheat plants of both varieties treated with fullerene C_{60}. Thus, the activity of catalase increased after treatment with fullerene C_{60} at high concentrations of 0.5 and 1.0 µg/ml by 125 and 150%, respectively, in plants of the Patras variety and by 150 and 173%, respectively, in plants of the Akter variety (Fig. 4).

The content of chlorophylls and MDA and the activity of antioxidant enzymes in plants are commonly used as markers of possible oxidative damage [69, 70]. An increase in chlorophyll content, MDA, and catalase activity in plants indicates oxidative damage. In addition, nanomaterials can cause changes in the physiological and biochemical properties and activity of antioxidant enzymes in biological systems [46, 71].

The increase in catalase activity may indicate both the negative effect of nanoparticles, namely excessive concentration of H_2O_2 in cells and the development of oxidative stress, and the protective properties of CNPs, since this enzyme splits hydrogen peroxide into water oxygen, thereby reducing lipid peroxidation [72].

Phenolic compounds play an important role in the antioxidant protection of cells from oxidative stress, providing protection of cell membranes and proteins [73, 74].

We observed the synthesis of phenolic compounds in wheat plants of the Patras variety treated with nanoparticles in the range of studied concentrations (0.1-1.0 µg/ml), namely, the content of phenolic compounds increased by 51, 58, 66 and 51%, respectively, compared to the control (Fig. 5), and in wheat plants of the Akter variety after exposure to 0.5 and 1.0 µg/ml of fullerene C_{60}, it increased by 21 and 38%, respectively, compared to the control. In addition, different sensitivity and adaptive properties of wheat varieties to the action of nanoparticles were observed. Thus, in the Akter variety, no increase in the content of phenolic compounds was detected under the action of fullerene C_{60} at low concentrations of 0.1 and 0.2 µg/ml, which may indicate greater stress resistance of the plants.

The results show the activation of the antioxidant defense, leading to the synthesis of phenolic compounds in wheat plants. Phenolic compounds neutralize reactive oxygen species generated under the action of C_{60} fullerene and play the role of signaling molecules in defense responses.

Thus, the intense synthesis of phenolic compounds indicates a protective response in plants to the stress induced by CNPs. Other authors have also shown that carbon nanomaterials, in particular nanotubes at moderate doses, as well as fullerene C_{60}, can act as elicitors (inducers of systemic resistance) in plants by increasing the content of phenolics [14].

Some studies have demonstrated the protective effects of CNPs against abiotic factors in plants. For example, treatment of seeds with 40 and 80 nM $C_{60}(OH)_{20}$ increased the content of flavanoids, phenolics, saccharides, amino acids and enhanced the

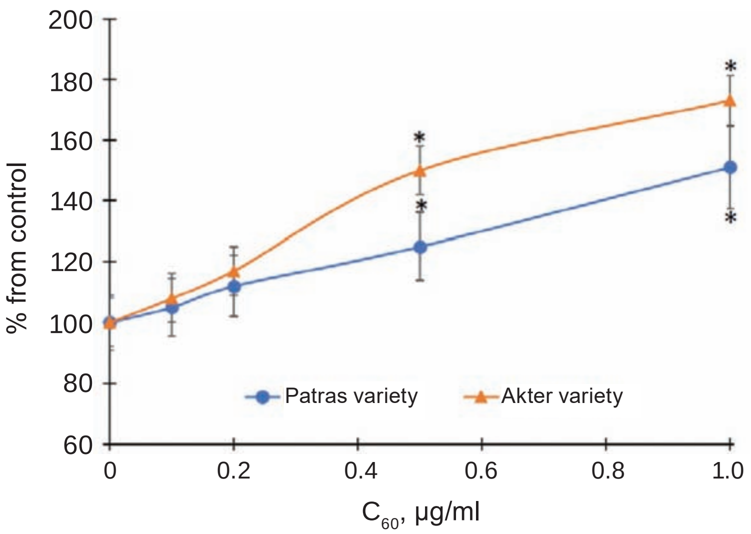

*Fig. 4. Change of catalase activity T. aestivum treated with fullerene C_{60}. *P < 0.05 in comparison with control (untreated plants)*

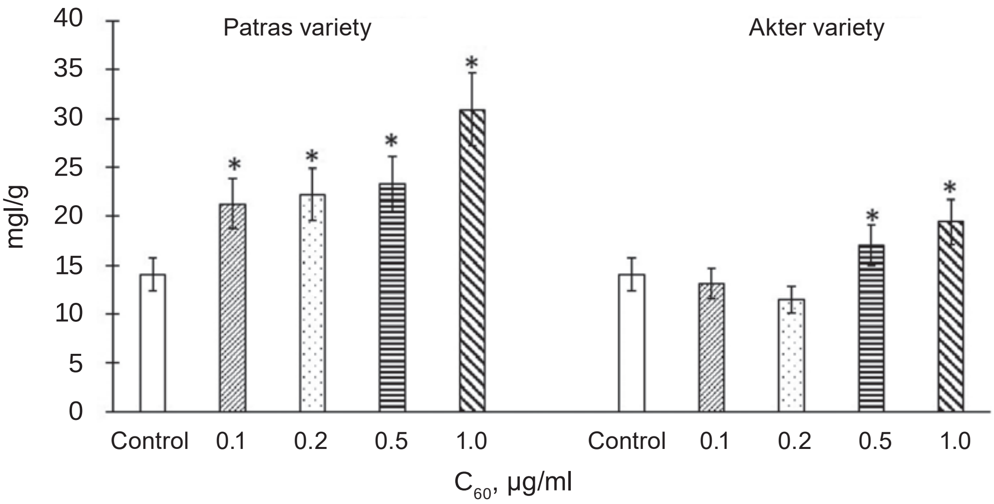

*Fig. 5. Phenolic compounds content (mg/g) in the leaves of wheat T. aestivum after treatment with fullerene C_{60}. *P < 0.05 in comparison with control (untreated plants)*

activity of catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) in wheat (*Triticum aestivum* L.) plants, while decreasing the content of MDA [75]. In addition, a positive and protective effect was observed in wheat plants subjected to six months of salt stress and foliar application of 40 and 80 nM $C_{60}(OH)_{20}$, accompanied by an increase in the content of chlorophylls, amino acids, and nutrients (calcium, potassium and phosphorus) [75].

When corn seeds (*Zea mays* L.) were treated with 5-50 mg/l $[C_{60}(OH)_{22}x8H_2O]_n$, a protective effect against drought was observed, as evidenced by increased activity of the antioxidant enzymes SOD and catalase, as well as a decrease in the content of H_2O_2 and MDA in seedlings [76]. Foliar treatment of sugar beet (*Beta vulgaris* L.) with 70 or 700 µmol $C_{60}(OH)_{24}$ was accompanied by an increase in proline content and the activity of catalase, ascorbate peroxidase, and glutathione peroxidase (EC 1.11.1.9) in roots and shoots under drought conditions [77].

Under conditions of foliar treatment of cucumber with 1.0 or 2.0 mg/l C_{60}, the concentration of chlorophyll-binding proteins (CAB), proton pumps (ATP synthase), photosynthetic protein complexes (PII), and cytochrome b6f increased. This led to improved light absorption, electron transfer (e–), synthesis of photosynthetic pigments and ATP, as well as enhanced activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glucose-6-phosphate isomerase (GPI) [78].

Conclusion. A dose-dependent effect of C_{60} fullerene was observed under seed treatment conditions, along with varying sensitivities of wheat varieties to the action of CNMs. It was shown that exposure to C_{60} fullerene resulted in an increase in plant biomass for the Patras wheat variety and root length for the Akter variety. At low doses (0.1-0.2 µg/ml), photosynthetic activity improved in plants of both wheat varieties, whereas at higher doses (0.5-1.0 µg/ml), it decreased, as evidenced by alterations in the content of photosynthetic pigments. Additionally, in wheat plants treated with C_{60} fullerene, protective systems were activated at both the membrane and cellular levels, as indicated by increased levels of MDA, phenolic compounds, and catalase activity. The ability of CNMs to activate the antioxidant potential in plants suggests promising applications in agrobiotechnology, which could aid in regulating stress tolerance and enhancing plant productivity. Nevertheless, further research is needed to elucidate the mechanisms underlying plant resistance and to establish adaptive responses to adverse growing conditions through the use of carbon nanomaterials.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ФІЗІОЛОГО-БІОХІМІЧНІ ПОКАЗНИКИ РОСЛИН ПШЕНИЦІ ОЗИМОЇ *TRITICUM AESTIVUM* L. ПІСЛЯ ОБРОБКИ НАСІННЯ ФУЛЕРЕНОМ C_{60}

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Екстремальні кліматичні умови, шкідники, хвороби та забруднення навколишнього середовища суттєво впливають на вирощування сільськогосподарської продукції та якість рослинної сировини. Припускається, що наноструктуровані вуглецеві матеріали, зокрема фулерен C_{60}, завдяки антиоксидантним, противірусним та антибактеріальним властивостям може бути використаний для запобігання цих впливів. Метою даного дослідження було оцінити вплив фулерену C_{60} за передпосівної обробки насіння пшениці на стан рослин через 14 днів після сходження. Насіння пшениці озимої *Triticum aestivum* L. сортів Патрас та Актер обробляли колоїдним розчином фулерену C_{60} (0,1-1,0 мкг/мл) упродовж 3 год. Біоморфометричні показники, вміст фотосинтетичних пігментів, фенольних сполук і МДА та активність каталази оцінювали за стандартними методиками. Показано, що після обробки насіння фулереном C_{60} значно збільшувалася як свіжа вага рослин пшениці сорту Актер, так і довжина пагонів сорту Патрас порівняно з необробленими контролями. Виявлено дозозалежну дію фулерену C_{60} на фізіолого-біохімічні показники рослин. Фотосинтетична активність у рослин обох сортів пшениці посилювалася після обробки насіння C_{60} за низьких концентрацій (0,1–0,2 мкг/мл), про що свідчить підвищення вмісту хлорофілів, а за високих концентрацій C_{60} (0,5–1,0 мкг/мл) знижувалася на фоні підвищення вмісту каротиноїдів. Спостерігалося посилення антиоксидантного захисту після обробки фулереном C_{60} за концентрацій 0,5–1,0 мкг/мл, про що свідчить підвищення вмісту фенольних сполук та активація каталази. Таким чином, обробка насіння пшениці фулереном C_{60} може розглядатися як перспективний підхід використання наночастинок вуглецю в агробіотехнологіях для покращення росту та стресостійкості рослин.

Ключові слова: проростання пшениці, обробка насіння, фулерен C_{60}, хлорофіли, каротиноїди, МДА, каталаза, фенольні сполуки.

References

1. Sadak MS. Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*). *Bull Nat Res Cent*. 2019; 43(1): 38.
2. Khan ST, Adil SF, Shaik MR, Alkhathlan HZ, Khan M, Khan M. Engineered Nanomaterials in Soil: Their Impact on Soil Microbiome and Plant Health. *Plants (Basel)*. 2021; 11(1): 109.
3. Deng Y, White JC, Xing B. Interactions between engineered nanomaterials and agricultural crops: Implications for food safety. *J Zhejiang Univ Sci A*. 2014; 15(8): 552-572.
4. Sharma MMM, Kapoor D, Loyal A, Kumar R, Sharma P, Husen A. Positive and Negative Impact of Carbon-Based Nanomaterials on the Plant Growth Performance. In: Husen A. (eds). Emerging Carbon Nanomaterials for Sustainable Agricultural Practices. Smart Nanomaterials Technology. Springer, Singapore, 2025. P. 63-83.
5. Tariq Z, Fatima A, Khurram S, Haider Z, Hassan S, Hussain MI, Anjum S.Influence of Carbon-Based Nanomaterials on Plant Cell Toxicity, Nutritional, and Active Compound Accumulation. In: Husen A. (eds). Emerging Carbon Nanomaterials for Sustainable Agricultural Practices. Smart Nanomaterials Technology. Springer, Singapore, 2025. P. 149-180.
6. Chaachouay N, Zidane L, Husen A. Carbon Nanomaterials for Healthier Crop Plants Growth and Enhanced Yield. In: Husen, A. (eds). Emerging Carbon Nanomaterials for Sustainable Agricultural Practices. Smart Nanomaterials Technology. Springer, 2025. P. 31-46.
7. Lin S, Reppert J, Hu Q, Hudson JS, Reid ML, Ratnikova TA, Rao AM, Luo H, Ke PC. Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small*. 2009; 5(10): 1128-1132.
8. De La Torre-Roche R, Hawthorne J, Deng Y, Xing B, Cai W, Newman LA, Wang C, Ma X, White JC. Fullerene-enhanced accumulation of

p,p'-DDE in agricultural crop species. *Environ Sci Technol*. 2012; 46(17): 9315-9323.
9. Avanasi R, Jackson WA, Sherwin B, Mudge JF, Anderson TA. C_{60} fullerene soil sorption, biodegradation, and plant uptake. *Environ Sci Technol*. 2014; 48(5): 2792-2797.
10. Liu Q, Chen B, Wang Q, Shi X, Xiao Z, Lin J, Fang X. Carbon nanotubes as molecular transporters for walled plant cells. *Nano Lett*. 2009; 9(3): 1007-1010.
11. Serag MF, Kaji N, Habuchi S, Bianco A, Baba Y. Nanobiotechnology meets plant cell biology: carbon nanotubes as organelle targeting nanocarriers. *RSC Advs*. 2013; 3(15): 4856-4862.
12. Giraldo JP, Landry MP, Faltermeier SM, McNicholas TP, Iverson NM, Boghossian AA, Reuel NF, Hilmer AJ, Sen F, Brew JA, Strano MS. Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nat Mater*. 2014; 13(4): 400-408.
13. Velikova V, Petrova N, Kovács L, Petrova A, Koleva D, Tsonev T, Taneva S, Petrov P, Krumova S. Single-walled carbon nanotubes modify leaf micromorphology, chloroplast ultrastructure and photosynthetic activity of pea plants. *Int J Mol Sci*. 2021; 22(9): 4878.
14. Sigala-Aguilar NA, López MG, Fernández-Luqueño F. Carbon-based nanomaterials as inducers of biocompounds in plants: Potential risks and perspectives. *Plant Physiol Biochem*. 2024; 212: 108753.
15. Nokandeh S, Ramezani M, Gerami M. The physiological and biochemical responses to engineered green graphene/metal nano-composites in *Stevia rebaudiana*. *J Plant Biochem Biotechnol*. 2021; 30: 579-585.
16. McGehee DL, Lahiani MH, Irin F, Green MJ, Khodakovskaya MV. Multiwalled carbon nanotubes dramatically affect the fruit metabolome of exposed tomato plants. *ACS Appl Mater Interfaces*. 2017; 9(38): 32430-32435.
17. Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V. The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. *Front Plant Sci*. 2021; 11: 552969.
18. Fatemi F, Abdollahi MR, Mirzaie-Asl A, Dastan D, Papadopoulou K. Phytochemical, antioxidant, enzyme activity and antifungal properties of Satureja khuzistanica *in vitro* and *in vivo* explants stimulated by some chemical elicitors. *Pharm Biol*. 2020; 58(1): 286-296.
19. Sharifi P, Bidabadi SS, Zaid A, Abdel Latef AAH. Efficacy of multi-walled carbon nanotubes in regulating growth performance, total glutathione and redox state of *Calendula officinalis L*. cultivated on Pb and Cd polluted soil. *Ecotoxicol Environ Saf*. 2021; 213: 112051.
20. Xiong JL, Ma N. Transcriptomic and meta-bolomic analyses reveal that fullerol improves drought tolerance in *Brassica napus* L. Int J Mol Sci. 2022; 23(23): 15304.
21. Zhang X, Cao H, Wang H, Zhang R, Jia H, Huang J, Zhao J, Yao J. Effects of graphene on morphology, microstructure and transcriptomic profiling of *Pinus tabuliformis* Carr. roots. *PLoS One*. 2021; 16(7): e0253812.
22. Hamdi H, De La Torre-Roche R, Hawthorne J, White JC. Impact of non-functionalized and amino-functionalized multiwall carbon nanotubes on pesticide uptake by lettuce (*Lactuca sativa* L.). *Nanotoxicology*. 2015; 9(2): 172-180.
23. Khodakovskaya MV, de Silva K, Biris AS, Dervishi E, Villagarcia H. Carbon nanotubes induce growth enhancement of tobacco cells. *ACS Nano*. 2012; 6(3): 2128-2135.
24. Lahiani MH, Dervishi E, Chen J, Nima Z, Gaume A, Biris AS, Khodakovskaya MV. Impact of carbon nanotube exposure to seeds of valuable crops. *ACS Appl Mater Interfaces*. 2013; 5(16): 7965-7973.
25. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. *Curr Opin Chem Biol*. 2005; 9(6): 674-679.
26. Torney F, Trewyn BG, Lin VS, Wang K. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nat Nanotechnol*. 2007; 2(5): 295-300.
27. Nima ZA, Lahiani MH, Watanabe F, Xu Y, Khodakovskaya MV, Biris AS. Plasmonically active nanorods for delivery of bio-active agents and high-sensitivity SERS detection in planta. *RSC Adv*. 2014; 4(110): 64985-64993.
28. Shen CX, Zhang QF, Li J, Bi FC, Yao N. Induction of programmed cell death in *Arabidopsis* and rice by single-wall carbon nanotubes. *Am J Bot*. 2010; 97(10): 1602-1609.
29. Begum P, Ikhtiari R, Fugetsu B, Matsuoka M, Akasaka T, Watari F. Phytotoxicity of multi-walled carbon nanotubes assessed by selected plant species in the seedling stage. *Appl Surf Sci*. 2012; 262: 120-124.

30. Shvedova AA, Pietroiusti A, Fadeel B, Kagan VE. Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. *Toxicol Appl Pharmacol*. 2012; 261(2): 121-133.
31. He A, Jiang J, Ding J, Sheng GD. Blocking effect of fullerene nanoparticles (nC_{60}) on the plant cell structure and its phytotoxicity. *Chemosphere*. 2021; 278: 130474.
32. Schuetze C, Ritter U, Scharff P, Fernekorn U, Prylutska S, Bychko A, Rybalchenko V, Prylutskyy Yu. Interaction of N-fluorescein-5-isothiocyanate pyrrolidine-C_{60} with a bimolecular lipid model membrane. *Mater Sci Eng C*. 2011; 31(5): 1148-1150.
33. Prylutska S, Grynyuk I, Skaterna T, Horak I, Grebinyk A, Drobot L, Matyshevska O, Senenko A, Prylutskyy Y, Naumovets A, Ritter U, Frohme M. Toxicity of C_{60} fullerene-cisplatin nanocomplex against Lewis lung carcinoma cells. *Arch Toxicol*. 2019; 93(5): 1213-1226.
34. Winter wheat Patras - DSV. Regime of access : https://www.dsv-ukraina.com.ua/sorte/664.
35. Production of soil mixtures, substrates and fertilizers for indoor and garden plants. Regime of access : http://floriada.com.ua/g_universal.php.
36. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem Soc Trans*. 1983; 11(5): 591-592.
37. Cakmak I, Horst WJ. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol Plant*. 1991; 83(3): 463-468.
38. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*. 1968; 125(1): 189-198.
39. Lucas BN, Dalla Nora, Boeira CP, Verruck S, da Rosa CS. Determination of total phenolic compounds in plant extracts via Folin-Ciocalteu's method adapted to the usage of digital images. *Food Sci Technol*. 2022; 42: e35122.
40. Luna CM, Pastori GM, Driscoll S, Groten K, Bernard S, Foyer CH. Drought controls on H_2O_2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. *J Exp Bot*. 2005; 56(411): 417-423.
41. Winter-hardy varieties of winter wheat. (n.d.). February 09, 2022. Regime of access: https://consumerhm.gov.ua/2966-zimostijki-sorti-ozimoji-pshenitsi.
42. Wheat seeds Akter DSV-Ukraine. Regime of access: https://superagronom.com/nasinnya-pshenicya-ozima/akter-id9728.
43. Al-Khayri JM, Rashmi R, Surya Ulhas R, Sudheer WN, Banadka A, Nagella P, Aldaej MI, Rezk AA, Shehata WF, Almaghasla MI. The role of nanoparticles in response of plants to abiotic stress at physiological, biochemical, and molecular levels. *Plants (Basel)*. 2023; 12(2): 292.
44. Prylutska SV, Franskevych DV, Yemets AI. Cellular biological and molecular genetic effects of carbon nanomaterials in plants. *Cytol Genet*. 2022; 56(4): 351-360.
45. Begum P, Fugetsu B. Phytotoxicity of multi-walled carbon nanotubes on red spinach (*Amaranthus tricolor* L) and the role of ascorbic acid as an antioxidant. *J Hazard Mater*. 2012; 243: 212-222.
46. Lahiani MH, Dervishi E, Ivanov I, Chen J, Khodakovskaya M. Comparative study of plant responses to carbon-based nanomaterials with different morphologies. *Nanotechnology*. 2016; 27(26): 265102.
47. Khodakovskaya MV, Kim BS, Kim JN, Alimohammadi M, Dervishi E, Mustafa T, Cernigla CE. Carbon nanotubes as plant growth regulators: effects on tomato growth, reproductive system, and soil microbial community. *Small*. 2013; 9(1): 115-123.
48. Buziashvili A, Prylutska S, Yemets A. Effect of fullerene C_{60} on tomato plants. *Innov Biosyst Bioeng*. 2024; 8(4): 13-22.
49. Li P, Xia Y, Song K, Liu D. The impact of nanomaterials on photosynthesis and antioxidant mechanisms in gramineae plants: research progress and future prospects. *Plants (Basel)*. 2024; 13(7): 984.
50. Newkirk GM, de Allende P, Jinkerson RE, Giraldo JP. Nanotechnology approaches for chloroplast biotechnology advancements. *Front Plant Sci*. 2021; 12: 691295.
51. Hu Z, Zhao C, Li Q, Feng Y, Zhang X, Lu Y, Ying R, Yin A, Ji W. Heavy metals can affect plant morphology and limit plant growth and photosynthesis processes. *Agronomy*. 2023; 13(10): 2601.

52. Yang Y, Zhang L, Huang X, Zhou Y, Quan Q, Li Y, Zhu X. Response of photosynthesis to different concentrations of heavy metals in *Davidia involucrata*. *PLoS One*. 2020; 15(3): e0228563.
53. Leonelli L, Erickson E, Lyska D, Niyogi KK. Transient expression in *Nicotiana benthamiana* for rapid functional analysis of genes involved in non-photochemical quenching and carotenoid biosynthesis. *Plant J*. 2016; 88(3): 375-386.
54. Taylor SH, Long SP. Slow induction of photosynthesis on shade to sun transitions in wheat may cost at least 21% of productivity. *Philos Trans R Soc Lond B Biol Sci*. 2017; 372(1730): 20160543.
55. Ashraf M, Harris PJ. Photosynthesis under stressful environments: An overview. *Photosynthetica*. 2013; 51(2): 163-190.
56. Romanenko KO, Babenko LM, Smirnov OE, Kosakivska IV. Antioxidant protection system and photosynthetic pigment composition in *Secale cereale* subjected to short-term temperature stresses. *Open Agric J*. 2022; 16(Suppl-1, M3): e187433152206273.
57. Smirnov O, Karpets LA, Zinchenko A, Kovalenko M, Belava V, Taran N. Changes of morphofunctional traits of *Triticum aestivum* and *Triticum dicoccum* seedlings caused by polyethylene glycol-modeling drought. *J Central Eur Agric*. 2020; 21(2): 268-274.
58. Maoka T. Carotenoids as natural functional pigments. *J Nat Med*. 2020; 74(1): 1-16.
59. Young AJ. The photoprotective role of carotenoids in higher plants. *Physiol Plant*. 1991; 83(4): 702-708.
60. Sun T, Rao S, Zhou X, Li L. Plant carotenoids: recent advances and future perspectives. *Mol Hortic*. 2022; 2(1): 3.
61. Horton P. Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. *J Exp Bot*. 2000; 51(Suppl 1): 475-485.
62. Horton P, Johnson MP, Perez-Bueno ML, Kiss AZ, Ruban AV. Photosynthetic acclimation: does the dynamic structure and macro-organisation of photosystem II in higher plant grana membranes regulate light harvesting states? *FEBS J*. 2008; 275(6): 1069-1079.
63. Souahi H. Impact of lead on the amount of chlorophyll and carotenoids in the leaves of *Triticum durum* and *T. aestivum*, *Hordeum vulgare* and *Avena sativa*. *Biosyst Divers*. 2021; 29(3): 207-210.
64. Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, Giordano G, Cucci MA, Graf M, Gentile F. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. *Antioxidants (Basel)*. 2018; 7(8): 102.
65. He Y, Wei HM, Liu SJ, Xu YC, Zhu ZY, Yan H, Li JX, Tian ZH. Growth response of *Oryza sativa* seedlings to graphene oxide and its variability among genotypes. *Biol Plant*. 2021; 65: 39-46.
66. Cheng F, Liu YF, Lu GY, Zhang XK, Xie LL, Yuan CF, Xu BB. Graphene oxide modulates root growth of *Brassica napus* L. and regulates ABA and IAA concentration. *J Plant Physiol*. 2016; 193: 57-63.
67. Zhang P, Guo Z, Luo W, Monikh FA, Xie C, Valsami-Jones E, Lynch I, Zhang Z. Graphene oxide-induced pH alteration, iron overload, and subsequent oxidative damage in rice (*Oryza sativa* L.): a new mechanism of nanomaterial phytotoxicity. *Environ Sci Technol*. 2020; 54(6): 3181-3190.
68. González-Gordo S, Rodríguez-Ruiz M, Palma JM, Corpas FJ. Comparative analysis of catalase activity in plants: spectrophotometry and native PAGE approaches. *Methods Mol Biol*. 2024; 2798: 213-221.
69. Hegedüs A, Erdei S, Horváth G. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Sci*. 2001; 160(6): 1085-1093.
70. Siddiqui MH, Al-Khaishany MY, Al-Qutami MA, Al-Whaibi MH, Grover A, Ali HM, Al-Wahibi MS. Morphological and physiological characterization of different genotypes of faba bean under heat stress. *Saudi J Biol Sci*. 2015; 22(5): 656-663.
71. Kovacic P, Somanathan R. Biomechanisms of nanoparticles (toxicants, antioxidants and therapeutics): electron transfer and reactive oxygen species. *J Nanosci Nanotechnol*. 2010; 10(12): 7919-7930.
72. Sairam RK, Rao KV, Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci*. 2002; 163(5): 1037-1046.

73. Chen D, Mubeen B, Hasnain A, Rizwan M, Adrees M, Naqvi SAH, Iqbal S, Kamran M, El-Sabrout AM, Elansary HO, Mahmoud EA, Alaklabi A, Sathish M, Din GMU. Corrigendum: Role of Promising Secondary Metabolites to Confer Resistance Against Environmental Stresses in Crop Plants: Current Scenario and Future Perspectives. *Front Plant Sci.* 2022; 13: 950612.
74. Muhammad M, Basit A, Wahab A, Li WJ, Shah ST, Mohamed HI. Response mechanism of plant stresses to secondary metabolites production. In: Fungal Secondary Metabolites. Elsevier, 2024. P. 469-492.
75. Shafiq F, Iqbal M, Ali M, Ashraf MA. Fullerenol regulates oxidative stress and tissue ionic homeostasis in spring wheat to improve net-primary productivity under salt-stress. *Ecotoxicol Environ Saf.* 2021; 211: 111901.
76. Liu F, Xiong F, Fan Y, Li J, Wang H, Xing G, Yan F, Tai F, He R. Facile and scalable fabrication engineering of fullerenol nanoparticles by improved alkaline-oxidation approach and its antioxidant potential in maize. *J Nanopart Res.* 2016; 18: 338.
77. Borišev M, Borišev I, Župunski M, Arsenov D, Pajević S, Ćurčić Ž, Vasin J, Djordjevic A. Drought impact is alleviated in sugar beets (*Beta vulgaris* L.) by foliar application of fullerenol nanoparticles. *PLoS One.* 2016; 11(11): e0166248.
78. Zhao L, Zhang H, Wang J, Tian L, Li F, Liu S, Peralta-Videa JR, Gardea-Torresdey JL, White JC, Huang Y, Keller A, Ji R. C_{60} fullerols enhance copper toxicity and alter the leaf metabolite and protein profile in cucumber. *Environ Sci Technol.* 2019; 53(4): 2171-2180.