Technical Physics, 2022, Vol. 67, No. 7

14

# Influence of nanocompositions based on light fullerene derivatives on cultural plants under favorable and stress conditions of their habitat

© G.G. Panova,[1] K.N. Semenov,[2] A.M. Artemieva,[1,3] E.A. Rogozhin,[4,5] A.S. Barashkova,[4] D.L. Kornyukhin,[3] Yu.V. Khomyakov,[1] E.V. Balashov,[1] A.S. Galushko,[1] V.E. Vertebnyi,[1] A.S. Zhuravleva,[1] E.N. Volkova,[1] A.M. Shpanev,[1] O.R. Udalova,[1] E.V. Kanash[1]

[1] Agrophysical Research Institute, St. Petersburg, Russia
[2] Pavlov First St. Petersburg State Medical University, St. Petersburg, Russia
[3] All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov, Saint Petersburg, Russia
[4] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia
[5] Gause Institute of New Antibiotics, Moscow, Russia
e-mail: gaiane@inbox.ru

*Received January 10, 2022*
*Revised January 10, 2022*
*Accepted March 1, 2022*

The relevance of the new forms of ecologically safe, stable in composition, in functional activity, biodegradable preparations development with a complex positive effect on plants remains high and the presented work is devoted to acquaintance with the generalized results of interdisciplinary studies for the effect on the system of soil (soil substitute) - cultivated plants by nanocompositions created based on carbon (water-soluble polyhydroxylated, carboxylated and amino acid derivatives of fullerene $C_{60}$) nanostructures with additions of trace elements in certain concentration ratios. Based on the previously identified concentration ranges of the tested fullerene derivatives with a positive effect on plants when treating their seeds, introducing them into the soil, other root habitats and foliar exposure, their compositions with salts of trace elements were developed and in a series of vegetation and field experiments under controlled favorable conditions and when modeling oxidative stress caused by UV-B irradiation, deficiency of soil moisture, deficiency of nutrients, phytopathogens, as well as in the natural conditions of the Leningrad region, the main mechanisms of positive impact (regulatory on vital systems, adaptogenic, immunomodulatory, protective) of the created nanocompositions on plants and the prospects of their application in plant growing are shown.

**Keywords:** nanocompositions, water-soluble fullerenes $C_{60}$, polyhydroxylated $C_{60}$, carboxylated $C_{60}$, amino acid derivatives C60, soil (soil substitute) — plant system, production process, abiotic stresses, resistance.

DOI: 10.21883/TP.2022.07.54485.344-21

## Introduction

Need in environmentally-friendly biodegradable preparations with stable complex positive effect on plants actualizes the search for and development of new form of the preparations and potential sources of such preparations — water-soluble fullerene derivatives. They are applicable in a wide variety of science and technology areas, including medicine and biology [1]. Thus, multiple investigations revealed antibacterial [2] and antiviral activities of fullerene derivatives, their antioxidant properties [3–8], complexing agent capability to ensure radioactive element delivery to target cells and to increase protozoa survival rate and resistance of animal and human organs and tissues to stress factors (gamma-irradiation, chemotherapy, etc.) [1,9–11], in a form of trend — to increase life span of laboratory animals due to oxidative stress suppression in their cells [12]. There are very few published research papers addressing the assessment of the influence of fullerene derivatives on agro- and ecosystems, living components, including plants [13–15]. And the information available is contradictory. Thus, various authors show that certain concentrations of polyhydroxylated fullerene are capable of damaging onion cells [16], facilitate the increase in green algae culture density *Pseudokirchneriella subcapitata,* arabidopsis hypocotyl growth acceleration [17], increase in bitter melon plant biomass by seed treatment with the polyhydroxylated fullerone solution, bitter melon yield gain and to increase the content of some useful substances in fruit [18]. The influence of fullerene derivatives like other nanomaterials is defined by their sizes, composition of functional groups, concentration, and species differences in plant response and environmental conditions [1,19,20]. Positive effect of the specified compounds on plants is probably associated with antioxidant activity, in particular with capability of binding reactive oxygen intermediates as was clearly shown on the barley plant example [21,22].

Aspects of fullerene derivative absorption, distribution, transformation or degradation in a plant have received almost no attention. Thus, the capability of absorbing and accumulating derivatives of fullerene $C_{60}$ or $C_{70}$, for example in rice, radish, onion, bitter melon and wheat is described in [13,14,16,18,23]. Water-soluble derivatives of fullerene $C_{60}$ penetrate through membrane of animal and plant origin or as lipophilic ions or in neutral form after protonation [23]. The example of wheat (*Triticum aestivum L.*) and radish (*Raphanus sativus L.*) germs shows that absorption of fullerene derivatives $C_{60}$ and $C_{70}$ by plants depends on fullerene derivative concentration in root-inhabited environment and these compounds are accumulated primarily in roots [14,24]. We know very little if nothing about the mechanisms of potential indirect influence of fullerene derivatives on plants after penetration soil. We were the first to show that increase in net production of plants and their resistance to oxidative stress after introduction of polyhydroxylated, carboxylated and aminoacid derivatives of $C_{60}$ in the root-inhabited environment or after foliar treatment of plants is evidently associated with the determined changes in the structure and efficiency of photosynthetic system and with the influence on the metabolic, substance exchange processes in plants, antioxidant protections systems, in particular on lipid peroxidation intensity, superoxide dismutase activity and reactive oxygen intermediate generation [21,25–28].

The purpose of the paper is to describe, on the basis of generalization of the obtained data, the basic features of the influence of nanocompositions containing water-soluble derivatives of light fullerenes $C_{60}$ and trace elements on cultivated plants and their habitat in controlled favourable conditions and in physical simulation of oxidative stress as well as in natural conditions of the Leningrad region.

## 1. Materials and methods

Water-soluble derivatives of fullerenes $C_{60}$ were the subject of research: polyhydroxylated fullerene (fullerenol), aminoacid fullerenes (with proline, hydroxiproline, threonine, glutamine, methionine, histidine, cysteine) and carboxylated fellirine (tris malonate) synthesized using a previously developed single-stage technique from individual fullerenes, fullerene mixture or fullerene soot using alkali water solution and phase transfer catalyst (tetra butyl ammonium hydroxide — TBAH) [29,30], and nanocomposition on the basis of synthesized fullerenes with trace elements solutions (know-how). Concentrations of fullerene derivatives $C_{60}$ are in the range: 0.001 to 1100 mg/l water.

To enhance protective functions of water-soluble derivatives of fullerene $C_{60}$, compositions with antimicrobial peptides have been developed: mixed solutions with various concentration ratios of tris malonate $C_{60}$ and thionine complex obtained from black cumin seeds (*Nigella sativa L.*) previously purchased in the Republic of Turkey and stored afterwards in a dark ventilated room at $+15-+18^{\circ}$C according to a previously tested algorithm, including acid extraction of mechanically ground seeds, reprecipitation of protein peptide fraction with acetone, desalination and three-stage fractionation by various liquid chromatography methods — pseudo-affinity and reverse-phase high performance liquid chromatography [31].

Cultivated test plants were used as the research object, including different varieties or hybrids of spring barley, soft wheat and some vegetable crops — water cress, lettuce, tomato, cucumber, etc., whose seeds were obtained from the collections of the All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov (VIR), Federal Research Center of Vegetable Growing and breeding/seed-production companies: „Gavrish“, AO SSPP „Sortsemovoshch“.

The research was carried out at the biological sites of the Agrophysical Research Institute in controlled natural conditions and in the VIR greenhouses. For plants cultivation at the Agrophysical Research Institute biological site in controlled microclimate conditions, original vegetation light system (VLS) prototypes were used [25,26,28].

For the assessment of fungicide and antibacterial activity of the tested nanomaterials and theirs nanocompositions, series of microvegetation tests to investigate the influence of the specified substances on the resistance of the test plants to fungal and bacterial phytopathogenic microorganisms infection were carried out using the procedures described in [32]. The following phytopathogens were used: a) cabbage black rot agent *Xanthomonas campestris* pv. *campestris,* strain 5212 (provided by the National Phytopathogenic Bacteria Collection, UK) as type strain of race 1 of cabbage black rot agent *Xanthomonas campestris* pv. *campestris*); b) fungal agents of dark-brown barley blotch and root rot *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur, cereal ear rot — *Alternaria* sp.; fusariosis — *Fusarium graminearum* Schwabe; dry rot of carrot — *Phoma rostrupii* Sacc (provided by the Genetic Department of the All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov).

The plants were grown within a set of vegetation experiments in favourable light, root-inhabited and air environment, and with physical simulation of oxidative stress action (soil moisture deficit, high-intensity UV-B irradiation), and in field experiments at agrobiological test site in the Gatchina district, Leningrad region. For vegetation experiments with physical soil moisture deficit simulation, Leningradskiy 6 and Opal spring wheat varieties were grown in vessels (volume of 3 $l$) with soddy podzolic soil in a permanently ventilated polycarbonate greenhouse with natural illumination, temperature and air humidity conditions and in controlled conditions at the Agrophysical Research Institute agrobiological test site, respectively. Soil moisture was kept at 60% of full water capacity by weighing the vessels on scales and wetting with designed amounts of water. During plant development — tillering phase–shooting phase — three foliar spray treatments were carried out at 5–7 day interval using nanocomposition solutions based on polyhydroxylated derivative of fullerene $C_{60}$, aminoacid derivative of fullerene $C_{60}$ with threonine, hydroxyproline, proline, and solutions of corresponding amino acids. Concentration of polyhydroxylated fullerene derivative was 15 mg/l; of aminoacid fullerene derivatives was — 0.1 mg/l each, aminoacid solutions had the same

**Table 1.** Evaluation of antimicrobial activity of mono and mixed solutions of $C_{60\text{-tm}}$ and thionine complex against fungal and bacterial phytopathogenic cultures of *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur and *Xanthomonas campestris* pv. *campestris,* strain 5212, respectively

| Substance concentrations, mg/l | | *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur | | *Xanthomonas campestris* pv. *campestris*, strain 5212 |
|---|---|---|---|---|
| $C_{60\text{-tm}}$ | Thionine complex | spawn growth suppression, 6th day, % | Presence/absence of colony growth (+/−), 5th day | Presence/absence of colony growth (+/−) |
| – | – | 0 | + | + |
| – | 0.001 | 0 | + | + |
| 0.01 | – | 0 | + | + |
| 0.1 | – | 0 | – | – |
| 0.01 | 0.001 | 0 | – | + |
| 0.1 | 0.001 | 0 | – | – |

concentrations as solutions with fullerene $C_{60}$ — threonine — 0.057 mg/l; proline — 0.024 mg/l; hydroxyproline — 0.027 mg/l.

To create stress conditions, 3 days after the last foliar treatment with nanocomposition solutions, moisture in some vessels was reduced from the favourable level — 60% of full water capacity (FWC) — to 25−30% of FWC and moisture deficit was kept at this level within 14 days, then the moisture level was increased in these options up to 60% of FWC. Plants treated with water and grown in soil with favourable moisture level 60% of FWC were used as control.

Vegetation tests for evaluation of the influence of the test derivatives of fullerene $C_{60}$ and nanocompositions thereof on the plant resistance to oxidative stress induced by high-intensity UV-B irradiation were carried out using the procedure described in [21,26].

Daily plant condition monitoring and phenological observations were carried out throughout the vegetation period. Upon completion of tillering−shooting period, including soil drought simulation stage, simultaneous measurements were carried out of soil moisture content by the gravimetric method; water potentials of plant leaves and roots using WP4-T Dewpoint Potentiometer, Decagon Devices, Inc [33,34].

Standard chemical and microbiological methods were used to assess quantitative characteristics of seed material and physiological condition of vegetative plants, changes in biological and chemical properties of soils, soil substitutes, behavior of related microorganisms [33–42].

Soil breathing was assessed in the vegetation experiment using sealed plastic chambers installed on the test soil surfaces. The chamber edges were inserted into soil at a depth of 0.5 cm. Gas phase volume in the chambers was 70 ml. At the end of incubation, a gas sample of 0.7 ml was taken from the chambers using a syringe. Content of $CO_2$ was measured in the samples immediately after sampling [38]. For this, 0.5 ml of gas was injected in LHM-8MD gas chromatograph with thermal conductivity detector. Hydrogen was used as a carrier gas.

Antioxidant system activity in plant roots and aerial parts was evaluated by lipid peroxidation (POL) intensity and peroxydase and catalase activity measurements. Lipid peroxidation was measured by malondialdehyde (MDA) accumulation in plants [39]. Peroxydase activity was determined by Boyarkin's photometric method with formation of benzidine blue [40]; catalase activity was determined by iodimetric method [41].

Standard procedures were used for chemical analysis of plants [33,37,41,42].

Upon completion of each vegetation test, plant growth biometrics were measured, including total weight and weight of organs — leaves, roots and stems. In tests where full life cycle of plants was used, major production parameters were evaluated.

Statistic data processing was carried out in Excel 2010 and Statistica 8 (Stat-Soft Inc., USA). Average values of the parameters in question and their confidence intervals were determined. Statistical significance of difference between the variants was evaluated by parametric statistical methods (Student's *t*-test criterion). Differences between the variants were considered significant at $p \leq 0.05$.

## 2. Research results

Preliminary laboratory and vegetation experiments before the main set of research detected, by test plant response, the concentration ranges of fullerene derivatives with positive, neutral and inhibitory action (seed or vegetative plant treatment) which were used to choose minimum positive-action concentrations of fullerene derivatives to be introduced into the root-inhabited environment and plant seed treatment for further investigation of test substance impacts on plants:

0.1, 1.0 and 10.0–15.0 mg/l and 0.1 mg/l for tris malonate fullerene $C_{60}$, aminoacid derivatives of fullerene $C_{60}$ and fullerenol $C_{60}$, respectively; for foliar treatment of vegetative plants — 0.01, 0.1 and 1.0–15.0 mg/l [21,25–28].

Polyhydroxylated, carboxylated and aminoacid derivatives of fullerene $C_{60}$ tested in these and wider concentration ranges did not have fungicide, fungistatic and/or antibacterial, bacteriostatic effects on some phytopathogen microorganisms: agents of cabbage black rot *Xanthomonas campestris* pv. *campestris;* cereal ear rot — *Alternaria* sp.; fusariosis — *Fusarium graminearum* Schwabe; dark-brown barley blotch and root rot — *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur; dry rot of carrot — *Phoma rostrupii* Sacc. At the same time, for aminoacid derivatives of fullerene $C_{60}$ with methionine or $C_{60}$ with threonine after seed treatment with their solutions in specific concentrations, capability of increasing spring barley resistance to root rot agent *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur was detected [32]. In this case, positive influence mechanism of these substances is not associated with control of number of microorganisms on seed surfaces and is apparently caused by comprehensive positive effect of fullerene derivatives on plants leading to improvement of their „immunity“. To enhance protective properties of nanocompositions on the basis of derivatives of fullerene $C_{60}$, we prepared mixed solutions of fullerene derivatives with antimicrobial peptides using *Nigella sativa* L seeds thionines. As an example, their concentrations were selected using which fungistatic effect was achieved against dark-brown barley blotch and root rot agent — fungus *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur, and antibacterial — against cabbage black rot *Xanthomonas campestris* pv. *campestris,* strain 5212. Thus, mixed test substance solutions of 0.001 mg/l thionine complex (hereinafter referred to as thionines) and 0.01 mg/l carboxylated derivative of fullerene $C_{60}$ ($C_{60\text{-tm}}$), and of 0.001 mg/l thionines and 0.1 mg/l $C_{60\text{-tm}}$ showed weak fungistatic properties, and mixed solution of 0.001 mg/l thionines and 0.1 mg/l $C_{60\text{-tm}}$ showed additional antibacterial properties, which indicate pronounced synergetic effect of these substances in mixed solutions in the specified concentrations, while thionine mono-solution at the same concentrations showed no restraining influence on the growth of fungus and bacterial pathogens, only 0.1 mg/l $C_{60\text{-tm}}$ mono-solution showed the influence similar to its mixed thionine solution (0.1 mg/l $C_{60\text{-tm}}$ and 0.001 mg/l thionine) (Table 1). According to the microvegetation experiments, the best ratio for joint treatment of watercress salad seeds with $C_{60\text{-tm}}$ and thionine solutions is 0.01 mg/l $C_{60\text{-tm}}$ with addition of 0.001 mg/l thionines. This treatment option shows the trend to increase in germination readiness and sprout length, and weak trend to reduce root length, which indicates the absence of negative effect of these acting substance concentrations on the watercress salad sprouts (data not shown) when comparing wet and dry weight of plants and dry substance content with the control. It is interesting to note that when the above ratio of $C_{60\text{-tm}}$ (0.01 mg/l) and thionines (0.001 mg/l) in the mixed solution was used for spring barley seeds treatment and subsequent germination in phytopathogen infected conditions — root rot agent *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur — the plants showed the highest resistance to this biotic stress (Table 2). It is manifested in larger amount of survived plants (90%), larger weight (by 25% in relation to the infected control) and in lower degree of disease development (score 2 on Goiman's scale [43]) compared with untreated control plants in infected conditions (score 3 on Goiman's scale [43]). It should be noted that although the other options of thionines and tris-malonate fullerene $C_{60}$ mixtures do not reduce the total lesion score of plants, these options show the trend to increase in the number of survived plants and their weight compared with the infected control (Table 2).

The set of vegetation experiments in controlled conditions using Leningradskiy spring barley variety showed that, for plants grown on soddy podzolic soil, introduction of nanocompositions based on aminoacid derivatives of fullerene $C_{60}$ with threonine or methionine, proline, hydroxyproline in the soil–plant system by means of foliar treatment of the latter had a significant impact on the number and activity of microorganisms in soil, while introduction of each substance of interest in the same amounts in soil did not caused significant changes in the number and activity of microbial community according to the release of $CO_2$ (Figure 1, Table 3). Thus, the option of foliar treatment of spring barley with nanocomposition solution based on aminoacid derivative of fullerene $C_{60}$ with threonine showed that content of $CO_2$ released from soil ($\mu$g/cm$^2$ soil per day) under the plants was 5.5 times higher than in the control (untreated plants), 3 times higher compared with water treatment and 2 times higher compared with trace elements solution treatment. Similar picture is also observed in case of foliar impact with nanocomposition solutions based on other aminoacid derivatives of fullerene $C_{60}$, but the manifestation of their effect is much lower.

Thus, activization of breathing in soddy podzolic soil under barley plants treated with nanocomposition solution indirectly indicates changes in metabolism and activization of exchange processes in plants which have significant effect on the activity and number of related microorganisms and processes performed by them. For example, significant increase in the number of proteolytic and amylolytic bacteria using easily-accessible organic and inorganic forms of nitrogen in their metabolism is observed in soil which is better represented in the option when plants are treated with nanocomposition solution based on aminoacid derivative of fullerene $C_{60}$ with methionine (Figure 1). Activization of microorganisms of the specified ecological-trophic groups in soil under the plants indicates intensification of nitrogen containing compound transformation processes in soil and potential improvement of nitrogen nutrition sources required for the plants which shall ultimately have a favorable effect on their physiological condition and growth, development, production processes.

**Table 2.** Influence of preplanting treatment of spring barley seeds with mono and mixed solutions of tris malonate fullerene $C_{60}$ ($C_{60\text{-tm}}$) and thionine complex on plant resistance to root rot agent *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur

| Substance concentrations, mg/l | Average number of survived plants | | Average weight of 1 plant | | Lesion score** |
|---|---|---|---|---|---|
| | pcs | % of control without infection | *g* | % of control without infection | |
| control ($H_2O$) (without infection) | 10.0 | 100 | 0.31 | 100 | 2 |
| control ($H_2O$) (with infection) | 7.0* | 70* | 0.16* | 52* | 3 |
| Thionines 0.001 | 7.2* | 72* | 0.17* | 55* | 3 |
| $C_{60\text{-tm}}$ 0.01 | 7.5* | 75* | 0.16* | 52* | 3 |
| $C_{60\text{-tm}}$ 0.1 | 8.0* | 80* | 0.17* | 55* | 3 |
| $C_{60\text{-tm}}$ 0.01+thionines 0.001 | 9.0 | 90 | 0.20* | 65* | 2 |
| $C_{60\text{-tm}}$ 0.1+thionines 0.001 | 8.5* | 85* | 0.18* | 58* | 3 |

Note. * — the value definitely differs from the control at 5% significance level;
** — to evaluate the lesion score, E. Goiman's scale was used [43].

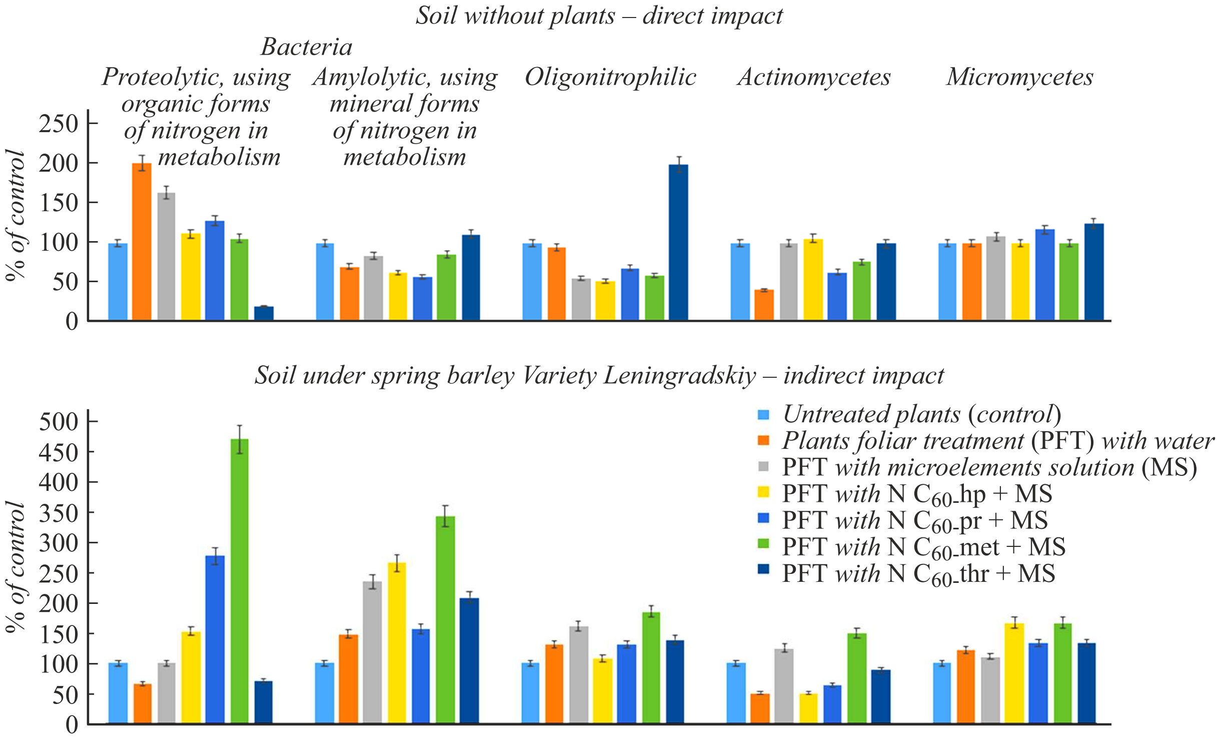

**Figure 1.** The number of microorganisms in soddy podzolic sandy-loam soil used to cultivate spring barley subjected to foliar treatment with nanocomposition solutions of the basis of aminoacid derivatives of fullerene $C_{60}$ and trace elements (in % of the control — water treated plants). MS — trace elements solution; N$C_{60\text{-hp}}$ + MS — nanocomposition solution based on fullerene $C_{60}$ with hydroxyproline and trace elements; N$C_{60\text{-pr}}$ + MS — nanocomposition solution based on fullerene $C_{60}$ with proline and trace elements; N$C_{60\text{-met}}$ + MS — nanocomposition solution based on fullerene $C_{60}$ with methionine and trace elements; N$C_{60\text{-thr}}$ + MS — nanocomposition solution based on fullerene $C_{60}$ with threonine and trace elements.

Actually, according to chemical analysis of soddy podzolic sandy-loam soil ( Ap layer), when nanocompositions based on derivatives of fullerene $C_{60}$ are directly introduced in soil and aerial part of plants is treated with solutions, soil is enriched with phosphorus, potassium, nitrogen as shown in Table 4 for aminoacid derivative of fullerene $C_{60}$ with threonine. In this case, the share of mobile forms of the specified macroelements and content of other macroelements and trace elements (data not shown) are increased. The above information shows that nanocompositions based of water-soluble derivatives of fullerene $C_{60}$ can increase macro- and trace elements accessibility in soil

**Table 3.** Change in content of $CO_2$ in chambers on soddy podzolic sandy-loam soil without plants after introduction of nanocomposition based on aminoacid derivative of fullerene $C_{60}$ with threonine and trace elements and on soil under spring barley subjected to foliar treatment with the specified nanocomposition

| foliar plant treatment option | Release of $CO_2$, $\mu$g/cm$^2$ soil per day | | | |
|---|---|---|---|---|
| | Absolute values | % of control | % of option with water treatment | % of option with MS treatment |
| Without treatment — control | Chambers on soil without plants | | | |
| | 0.174 | 100 | 32* | 64* |
| Water | 0.538* | 309* | 100 | 199* |
| PM | 0.271* | 156* | 50* | 100 |
| NFthr + MS | 0.449* | 258* | 84* | 166* |
| Without treatment — control | Chambers on soil under plants | | | |
| | 0.514 | 100 | 54* | 36 |
| Water | 0.945* | 184* | 100 | 67 |
| PM | 1.420* | 276* | 150* | 100 |
| NFthr + MS | 2.839 | 552* | 300* | 200 |

Note. MS — macroelements and trace elements solution; NFthr + MS — nanocoposition solution based on fullerene $C_{60}$ with threonine and trace elements; * — the value definitely differs from the control at 5% significance level; nanocomposition and foliar treatment were introduced simultaneously three times during barley shooting period. Incubation of chambers installed on soil in vessels lasted 2 days.

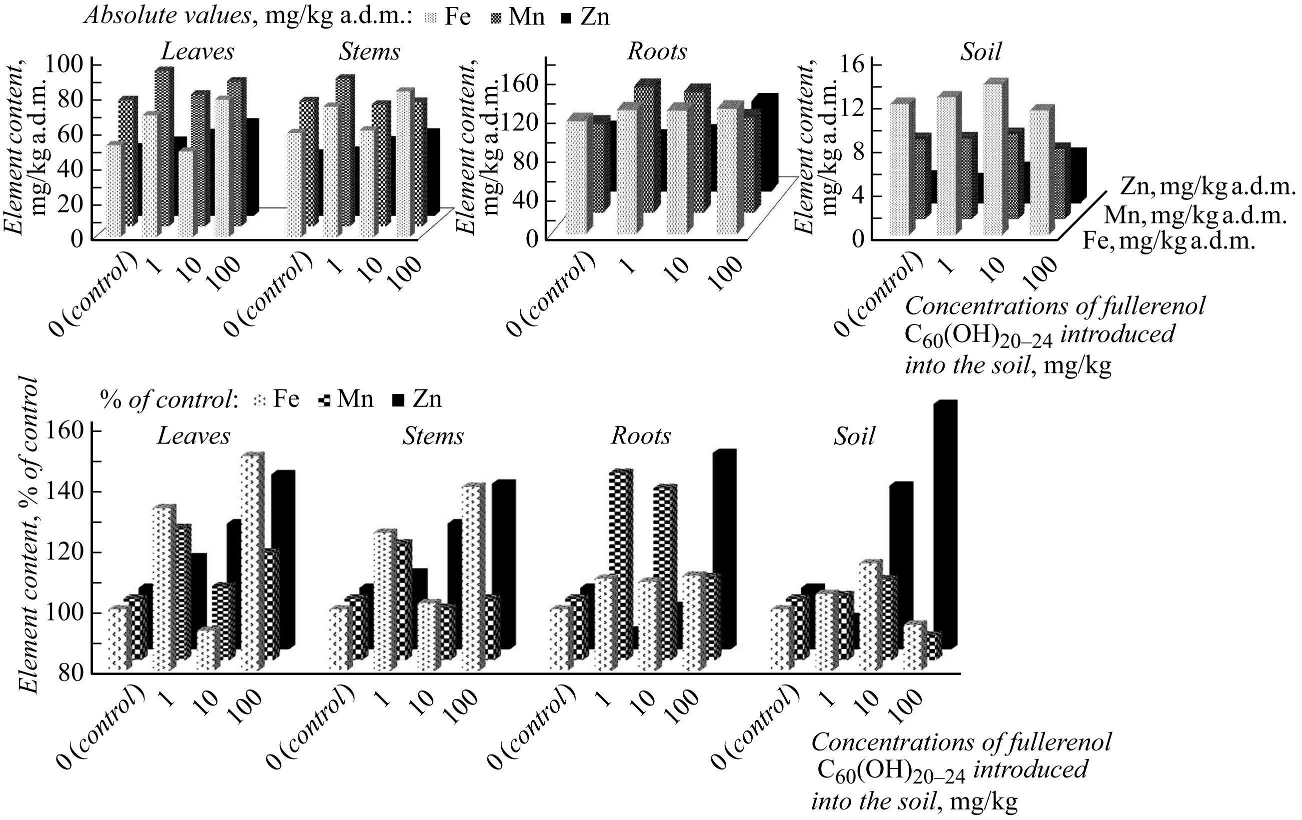

**Figure 2.** Impact of introduction of fullerenol $C_{60}(OH)_{20-24}$ solutions in various concentrations in soil on the content of studied trace elements in soil and cucumber plants (hybrid $F_1$ Neva). * — value × $10^1$, a.d.m. — absolutely dry matter.

**Table 4.** Content of carbon, main macroelements and active nitrogen forms in soddy podzolic sandy-loam soil, in roots and aerial part of spring barley when nanocomposition based on fullerene $C_{60}$ with threonine and trace elements is introduced in soil and used for foliar treatment of plants

| Test options | Substance content | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $C_{tot}$, % | Carbon (aqueous extract), % | Phosphate, ion, mg/kg | potassium, mg/kg | total nitrogen, % | Ammonia nitrogen, mg/kg | Nitrate nitrogen, mg/kg | pH, units |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| In soil with solutions of test substances introduced in it | | | | | | | | |
| Control (water) | 1.20 | 0.32 | 458 | 104 | 0.25 | 8.2 | 25.4 | 5.72 |
| Trace elements solution (control) | 1.11 | 0.34 | 670* | 167* | 0.27 | 9.3* | 32.6* | 5.71 |
| Fullerene $C_{60}$ with threonine ($C_{60\,thr}$) | 1.63* | 0.43* | 720* | 109 | 0.25 | 10.3* | 16.4* | 5.68 |
| Nanocomposition based on fullerene $C_{60\,thr}$, trace elements | 1.68* | 0.46* | 950* | 210* | 0.24 | 12.1* | 16.0* | 5.70 |
| In soil with foliar treatment of plants with solutions of test substances | | | | | | | | |
| Control (water) | 1.09 | 0.37 | 820 | 130 | 0.28 | 16.8 | 32.0 | 5.64 |
| Trace elements solution (control) | 1.00 | 0.42 | 1400* | 210* | 0.36* | 27.2* | 37.6* | 5.58 |
| Fullerene $C_{60\,thr}$ | 1.23* | 0.48* | 2300* | 176* | 0.39* | 18.6 | 19.8* | 5.54 |
| Nanocomposition based on fullerene $C_{60\,thr}$, trace elements | 1.34* | 0.54* | 4190* | 730* | 0.74* | 30.2* | 23.6* | 5.56 |
| In plant roots with solutions of test substances introduced in soil | | | | | | | | |
| Control (water) | – | 1.02 | 0.92 | 0.99 | 0.51 | – | 204.5 | – |
| Trace elements solution (control) | – | 2.03* | 1.11* | 2.23* | 0.47 | – | 200.1 | – |
| Fullerene $C_{60\,thr}$ | – | 3.02* | 1.14* | 3.01* | 0.62* | – | 198.2 | – |
| Nanocomposition based on fullerene $C_{60\,thr}$, trace elements | – | 4.20* | 1.40* | 4.27* | 0.59* | – | 225.3 | – |
| In aerial part with solutions of test substances introduced in soil | | | | | | | | |
| Control (water) | – | 0.99 | 0.62 | 1.70 | 1.20 | – | 224.5 | – |
| Trace elements solution (control) | – | 1.92* | 0.71 | 3.12* | 1.23 | – | 210.1 | – |
| Fullerene $C_{60\,thr}$ | – | 2.96* | 0.74 | 5.27* | 1.76* | – | 232.4 | – |
| Nanocomposition based on fullerene $C_{60\,thr}$, trace elements | – | 3.18* | 0.90* | 6.50* | 2.06* | – | 240.1 | – |
| In plant roots with foliar treatment of plants with solutions of test substances | | | | | | | | |
| Control (water) | – | 1.12 | 0.99 | 1.12 | 0.33 | – | 231.0 | – |
| Trace elements solution (control) | – | 1.01 | 0.84 | 0.98 | 0.30 | – | 220.0 | – |
| Fullerene $C_{60\,thr}$ | – | 3.57* | 1.07 | 3.27* | 0.37 | – | 218.2 | – |
| Nanocomposition based on fullerene $C_{60\,thr}$, trace elements | – | 3.46* | 0.98 | 2.99* | 0.37 | – | 221.4 | – |
| In aerial part of plants with foliar treatment of plants with solutions of test substances | | | | | | | | |
| Control (water) | – | 0.80 | 0.60 | 1.68 | 1.00 | – | 272.4 | – |
| Trace elements solution (control) | – | 0.88 | 0.67 | 1.89* | 1.20* | – | 252.1 | – |
| Fullerene $C_{60\,thr}$ | – | 2.73* | 0.66 | 4.72* | 1.28* | – | 264.9 | – |
| Nanocomposition based on fullerene $C_{60\,thr}$, trace elements | – | 2.90* | 0.69 | 4.84* | 1.28* | – | 295.1 | – |

Note. * — value is definitely different from the control at 5% significance level.

for plants. This assumption is supported by the chemical analysis of spring barley (Table 4, Figure 2). In roots and aerial part of plants subjected to foliar impact with nanocomposition solutions based on fullerene derivatives or grown in soil where the same nanocompositions were introduced, the highest content of macro- and trace elements is observed compared with the control (water treatment of soil and plants) and with macro- and trace elements solution treatment options.

The characterized ability of nanocompositions based on derivatives of fullerene $C_{60}$ to intensify useful macro- and trace elements absorption and transport in aerial part of plants when their solutions are introduced in soil allowed to suggest potential ability of the created composition to minimize the consequences of deficit of some trace elements in root-inhabited environment for plants, including positively charged zinc and manganese cations which are known to play important role in metabolism, photosynthesis, breathing processes, etc. Due to low mobility of zinc and manganese compounds in soil (especially in carbonate and chalky), development of new approaches to plant nutrition optimization using these trace elements is a rather important task and many research laboratories worldwide foster their efforts to fulfil it. The above assumption was proved by a set of vegetation experiments. Thus, the investigations carried out in controlled conditions for growing hybrid $F_1$ Neva cucumber in aerated cultural solutions not containing manganese or zinc, and with double spraying of plants (2nd and 4th true leaf stages) with magnesium or zinc sulphate, fullerenol $C_{60}$ in two concentrations (1 and 2 mg/l), mixed magnesium or zinc sulphate and fullerenol $C_{60}$ in the specified concentrations showed the ability of fullerenol $C_{60}$ introduced by foliar applications to influence actively on the manganese and zinc accessibility for plants and accumulation (Table 5 and 6). In options with application of the specified mixed solutions with increased number of treatments, the specified positive effects are enhanced and, for example, manganese content on the 7th day after the second foliar treatment or zinc content in leaves on the 3rd day in these options definitely exceeds quantitative values of the specified trace elements in the manganese or zinc sulphate treatment option in addition to the quantitative values in the control.

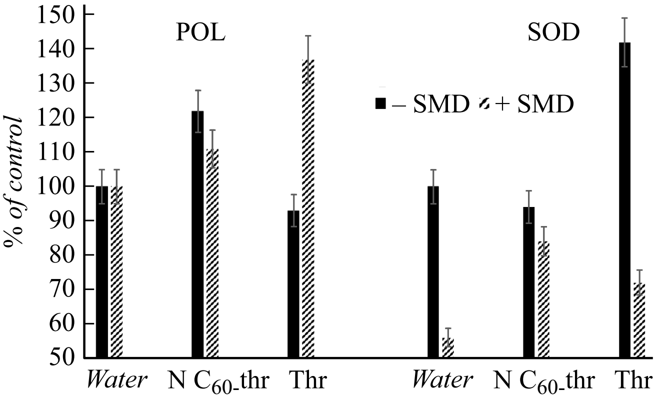

**Figure 3.** Influence of foliar treatment of Leningradskiy spring soft wheat variety 6 with nanocomposition solutions based on aminoacid derivative of fullerene $C_{60}$ with threonine and threonine aminoacid on antioxidant system activity parameters (POL intensity and SOD activity) of plants grown in controlled favourable conditions and under stress induced by soil moisture deficit (25–30% of full water capacity of soil). N $C_{60\text{-thr}}$ — nanocomposition based on aminoacid derivative of fullerene $C_{60}$ with threonine; Thr — threonine aminoacid; SMD — soil moisture deficit; POL — lipid peroxidation intensity; SOD — superoxide dismutase activity.

Intensification of macro- and trace elements delivery into plants and, in particular with leaves in options with introduction of fullerene derivatives and nanocompositions thereof causes activization of metabolism processes in plants, including biosynthesis of photosynthetic pigments. For different cultures, a common similar pigment content change pattern was observed under the effect of nanocompositions based on water-soluble derivatives of fullerene $C_{60}$. This can be observed more clearly and vividly in cabbage cultures (Table 7) in whose leaves, under the effect of nanocompositions based on aminoacid derivatives with glutamine, methionine, threonine, proline, histidine, significant increase in content of chlorophyll *a* and *b* as well as carotenoid pigments and carotene also actively involved in oxidation-reduction reactions and known to have adaptogenic, antioxidant and protective properties [44–46].

We have already discussed the ability of fullerene derivatives and nancompositions thereof to influence directly or indirectly antioxidant properties of plants and to bind free radicals in plant cells [21,25,26,28]. Thus, we demonstrated that when root tips preliminary treated with the specified derivatives of fullerene $C_{60}$ were exposed to UV-B irradiation (0.5 W/m$^2$, 15 min), their ability to reduce formation of free radicals and inhibit development of oxidative stress in plant root cells is pronounced [21,25,26]. According the change in activity of peroxydase, catalase, superoxide dismutase involved in oxidation-reduction reactions in plant cells and tissues, and lipid peroxidation intensity, reactive oxygen intermediate content in plants treated with solutions of fullerene $C_{60}$ derivatives and nanocompositions thereof, stabilization of antioxidant systems of plants is observed and is more vividly manifested in oxidative stress conditions caused by UV-B irradiation, soil moisture deficit, manganese, zinc and other trace elements content deficit in root-inhabited environments, etc. [25–28]. Thus, Leningradskiy 6 soft spring wheat exposed to foliar treatment with nanocomposition solutions based on aminoacid derivative of $C_{60}$ with threonine during tillering-shooting and then to two-week water stress shows that variation amplitude of oxidation-reduction superoxide dismutase activity, lipid peroxidation intensity and, therefore, development of oxidative stress in them is significantly lower compared with that observed in control plants and treated with threonine solution in soil moisture deficit options (Figure 3). This is apparently associated with previously revealed effect of water potential increase in plant leaves under the effect of fullerene

**Table 5.** Impact of manganese deficit in root-inhabited environment and foliar threatment with mono- and mixed solutions of fullerenol $C_{60}$ (Fd) and/or magnesium sulphate on the content of the tested trace elements in cucumber leaves (hybrid $F_1$ Neva) when plants are grown in controlled conditions of intensive photoculture

| Foliar option treatment of plants | 2nd day after the first foliar treatment of plants | | | 7th day after the first foliar treatment of plants | | | 7th day after the second foliar treatment of plants | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fe, mg/kg d.s. | Mn, mg/kg d.s. | Zn, mg/kg d.s. | Fe, mg/kg d.s. | Mn, mg/kg d.s. | Zn, mg/kg d.s. | Fe, mg/kg d.s. | Mn, mg/kg d.s. | Zn, mg/kg d.s. |
| Absolute values with manganese deficit | | | | | | | | | |
| Control (distilled water) | 71 ± 2f | 91 ± 2c | 81 ± 1e | 52 ± 2c | 91 ± 5b | 83 ± 1d | 71 ± 2f | 68 ± 1d | 57 ± 1b |
| −Mn | 56 ± 1d | 30 ± 1a | 48 ± 1a | 61±2d | 15 ± 1a | 44 ± 0.5c | 56 ± 1d | 17 ± 1a | 64 ± 1c |
| $MnSO_4$ | 47 ± 1a | 207 ± 3e | 64 ± 1c | 38 ± 1a | 141 ± 5d | 32 ± 0.5a | 47 ± 1a | 127 ± 1e | 36 ± 1a |
| Fd1 | 55 ± 3cd | 89 ± 2c | 89 ± 4f | 44 ± 2b | 19 ± 0.5a | 37 ± 1b | 54 ± 3cd | 25 ± 0.5c | 64 ± 3c |
| Fd2 | 66 ± 2e | 46 ± 3b | 62 ± 1c | 52 ± 1c | 16 ± 1a | 43 ± 2c | 66 ± 2e | 22 ± 1b | 53 ± 1b |
| $MnSO_4$+Fd1 | 49 ± 2ab | 200 ± 2d | 54 ± 2b | 44 ± 1b | 107 ± 5c | 34 ± 1a | 49 ± 2ab | 187 ± 1f | 72 ± 4d |
| $MnSO_4$+Fd2 | 51 ± 3bc | 277 ± 8f | 73 ± 3d | 44 ± 0.5b | 107 ± 2c | 38 ± 0.5b | 51 ± 3bc | 286 ± 1j | 65 ± 4c |
| % of control with manganese deficit | | | | | | | | | |
| −Mn | 79d | 33a | 59a | 117d | 16a | 53c | 79d | 25a | 112c |
| $MnSO_4$ | 66a | 227e | 79c | 73a | 155d | 39a | 66a | 187e | 63a |
| Fd1 | 77cd | 98c | 110f | 85b | 21a | 45b | 76d | 37c | 112c |
| Fd2 | 93e | 51b | 77c | 100c | 18a | 52c | 93e | 32b | 93b |
| $MnSO_4$+Fd1 | 69ab | 220d | 67b | 85b | 118c | 41a | 69b | 275f | 126d |
| $MnSO_4$+Fd2 | 72bc | 304f | 90d | 85b | 118b | 46b | 72c | 421j | 114c |

Note. Average values are given ±SD. equivalent letters denote the absence of definite differences between average values (criterium SNK, $P < 0.05$). Fd1 — 1 mg/l fullerenol solution; Fd2 — 2 mg/l fullerenol solution; d.s. — dry substance.

derivatives (Figure 4) caused, in turn, by significant increase in content of macro- and trace elements in plant leaves and, consequently, probably osmotic pressure in their cells that ensured more efficient water delivery in them compared with the control plants in stress conditions. Provision of plants in stress conditions with accessible resources for formation of protective substances and stable operation of all plant systems also prevented negative impact of soil moisture deficit on growth, development and production levels. In option with foliar treatment with nanocomposition solution based on aminoacid derivative of fullerene $C_{60}$ with threonine, grain production exceeds by 96% that of the control plants not subjected to stress (Table 8). It should be noted that treatment of plants with threonine solution also facilitated their resistance to soil moisture deficit in a form of trend (grain production is by 7% higher than the control values, respectively), but the manifestation of the effect was significantly lower compared with the influence of nanocomposition based on fullerene $C_{60}$ with threonine and macro- and trace elements. It is also interesting to note the redistribution of macroelements into wheat grains due to reduced weight of non-productive part under the influence nanocomposition. Thus, the analysis of spring wheat straw weight vs. grain weight and straw element composition vs. grain element composition has shown that foliar treatment with nanocomposition based on fullerene $C_{60}$ with threonine ensures redistribution of plastic substances and inorganic nutrients into grain (Table 8). This effect of the specified nanocomposition is especially pronounced in stressor (drought) impact conditions. Nitrogen fixation by wheat grain in this option compared with that in the control plant without stress and in the control plant with stress is 2.8 and 3.9 times higher, respectively; phosphorus is 1.9 and 4.1 times higher; potassium is 1.2 and 2.8 times higher. Such pronounced positive effect of threonine-containing fullerene may be associated with more multi-purpose use of threonine as an intermediate product in various synthesis routes of protective substances exposed to stressors. Thus, it is known that the product of threonine transformation to leucine or valine is pyruvate (see, for example [47]) which is transformed to acetyl coenzyme A in aerobic conditions, which, in turn, serves as the main substrate for a set of reactions known as the Krebs cycle with subsequent formation of various secondary metabolites with protective, signal and other functions.

As we have found earlier, fullerene derivatives ensure intensification of macro- and trace elements delivery to plants and are able to penetrate into plants themselves, and

**Table 6.** Impact of zinc deficit in root-inhabited environment and foliar threatment with mono- and mixed solutions of fullerenol $C_{60}$ (Fd) and/or magnesium sulphate on the content of the test trace elements in cucumber leaves (hybrid $F_1$ Neva) when plants are grown in controlled conditions of intensive photoculture

| Foliar plant treatment option | 3rd day after the first foliar plant treatment option | | |
|---|---|---|---|
| | Fe, mg/kg d.s. | Mn, mg/kg d.s. | Zn, mg/kg d.s. |
| Absolute values | | | |
| Control | $59 \pm 1$c | $83 \pm 1$f | $40 \pm 2$c |
| $-$Zn | $45 \pm 2$a | $47 \pm 0.5$b | $18 \pm 1$a |
| $ZnSO_4$ | $51 \pm 1$b | $48 \pm 1$bc | $92 \pm 3$d |
| Fd1 | $49 \pm 1$b | $50 \pm 2$c | $24 \pm 0.5$b |
| Fd2 | $41 \pm 3$a | $41 \pm 1$a | $21 \pm 0.5$ab |
| $ZnSO_4$+Fd1 | $56 \pm 3$c | $62 \pm 2$e | $137 \pm 4$e |
| $ZnSO_4$+Fd2 | $61 \pm 2$c | $59 \pm 1$d | $153 \pm 1$f |
| % of control | | | |
| $-$Zn | 76a | 57b | 45a |
| $ZnSO_4$ | 86b | 58bc | 230d |
| Fd1 | 83b | 60c | 60b |
| Fd2 | 69a | 49a | 53ab |
| $ZnSO_4$+Fd1 | 95c | 75e | 343e |
| $ZnSO_4$+Fd2 | 103c | 71d | 383f |

Note. Average values are given ±SD. Equivalent letters denote the absence of definite differences between average values (criterium SNK, $P < 0.05$). Fd1 — 1 mg/l fullerenol solution; Fd2 — 2 mg/l fullerenol solution.

are found in large amounts in that organ through which they have been delivered into the plants [28].

The revealed set of mechanisms of positive effect of water-soluble fullerene derivatives and nanocompositions thereof on plants most likely caused significant increase in resistance of two spring barley varieties to epiphytoties in field experiments and the observed significant increase in case of combined use of efficient application of chemical fungicides in half doses — to the levels close to the effects of these agrochemicals in full doses [26], which opens perspectives of reduction of pesticide doze budget load on agro-ecosystems.

Thus, summing up the results of comprehensive interdisciplinary research, conclusion can be made regarding the following main mechanisms of impacts of the developed nanocompositions based on water-soluble derivatives of fullerene $C_{60}$ in plants:

— antioxidant properties — ability to prevent oxidative stress development in plant organs by direct interaction with free radicals as well as activization of metabolic processes in plants leading to neutralization of compounds containing radical groups;

— regulatory impact on the active operation of photosynthetic system, primary and secondary metabolism processes with enhanced formation and/or preservation of chlorophyll content, and compounds with adaptogenic, antioxidant and protective properties (flavonols, carotenoids, etc.), activization of ferments (catalase, superoxide dismutase and peroxydase) capable of neutralizing free radicals;

— direct (via penetration in soil) and indirect (through plant) significant regulatory impact of various taxonomic and ecological-trophic group on the number and activity of microorganisms, biological and chemical processes in soil, quantitative composition of main macroelements and their active forms;

— absence of fungicide and antibacterial properties in the majority of tested polyhydroxylated, aminoacid derivatives of fullerene $C_{60}$ in entire concentration range optimal for plant growth and development in relation of several key fungal and bacterial phytopathogens. Only synthesized carboxylated derivative of fullerene $C_{60}$ — trismalonate fullerene $C_{60}$ and its composition with antimicrobial polypeptides from thionine structural family in certain concentrations have shown weak fungistatic and antibacterial ability against the specified pathogenes;

— regulatory impact on transport and redistribution of nutrients over plant organs, i.e. enhanced macro- and trace elements delivery to plants; stimulation of nutrient attraction

**Table 7.** Biochemical composition of Da-Tsin-Kou Chinese cabbage after seed treatments with test substance solutions (10 mg/l) when plants are grown in VIR greenhouse

| Seed treatment option | Dry weight, % | Ascorbic acid , mg/100 g | chlorophyll A, mg/100 g | chlorophyll B, mg/100 g | chlorophylls, mg/100 g | carotenoids, mg/100 g | carotenes, mg/100 g | $\beta$-carotene, mg/100 g |
|---|---|---|---|---|---|---|---|---|
| | % of control | | | | | | | |
| Water (control) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Heteroauxin | 91 | 106 | 70* | 72* | 71* | 63* | 69* | 67* |
| NFcys | 76* | 113 | 85* | 98 | 88 | 93 | 107 | 93 |
| NFhp | 100 | 106 | 100 | 108 | 102 | 85* | 105 | 96 |
| NFglu | 100 | 100 | 130* | 145* | 134* | 138* | 168* | 139* |
| NFmet | 88 | 100 | 136* | 140* | 137* | 132* | 156* | 137* |
| NFpro | 102 | 106 | 113* | 127* | 117* | 134* | 144* | 129* |
| NFthr | 102 | 100 | 117* | 128* | 120* | 103 | 131* | 114* |
| NFhis | 106 | 106 | 118* | 126* | 120* | 129* | 178* | 124* |
| Cysteine | 111 | 113 | 100 | 107 | 102 | 82* | 95 | 95 |
| Hydroxyproline | 86* | 106 | 91 | 96 | 93 | 123* | 110 | 108 |
| Glutamine | 77* | 100 | 73* | 71* | 72* | 74* | 73* | 71* |
| Methionine | 90 | 100 | 90 | 97 | 92 | 73* | 87* | 86* |
| Proline | 88 | 113 | 97 | 110 | 101 | 106 | 104 | 108 |
| Threonine | 82* | 106 | 102 | 112 | 105 | 104 | 107 | 108 |
| Histidine | 84* | 106 | 116* | 136* | 121* | 96 | 114* | 118* |

Note. * — the value definitely differs from the control at 5% significance level; NFaminoacid — nanocomposition based on fullerene $C_{60}$ with aminoacid (cys — cysteine, hp — hydroxiproline, glu — glutamine, met — methionine, pro — proline, thr — threonine, his — histidine).

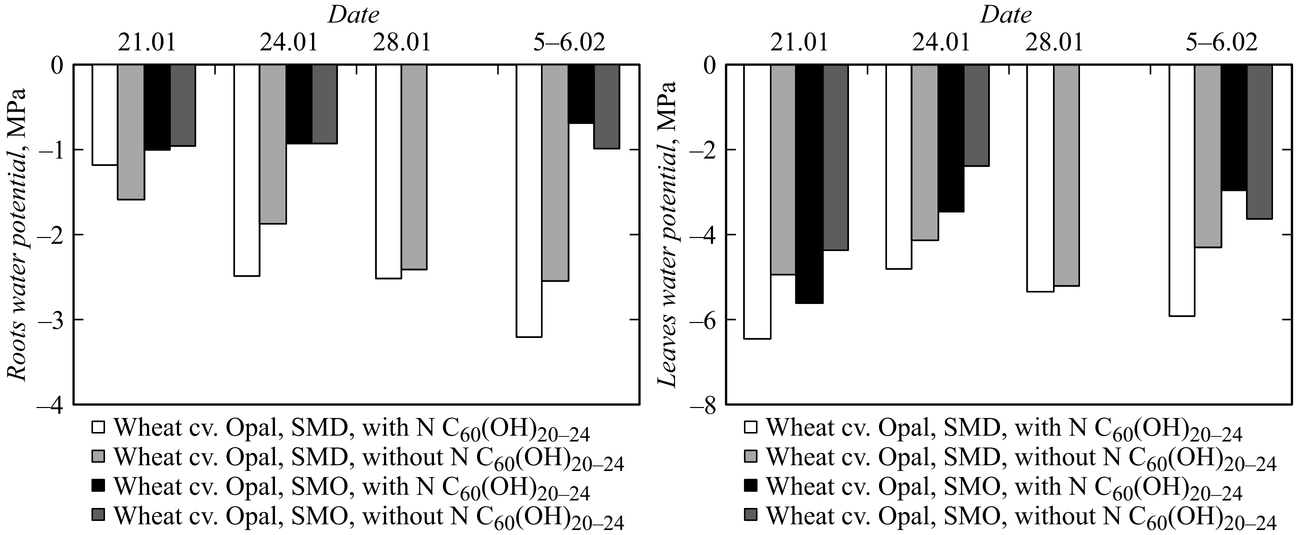

**Figure 4.** Dynamics of water potentials of roots and leaves of Opal soft spring wheat variety after foliar treatment with nanocomposition solution based on polyhydroxylated fullerene $C_{60}$ (F) in deficit and optimum soil moisture content conditions. SMD — soil moisture deficit (soil moisture — 25–30% of full water capacity), SMO — optimum soil moisture content (soil moisture — 60% of full water capacity).

**Table 8.** Production and depletion of main elements by grain of Leningradskiy 6 spring wheat variety with foliar treatment of it vegetative plants with nanocompositions based on aminoacid derivative of fullerene $C_{60}$ with threonine or threonine solution in favourable and stress conditions in terms of soil moisture content and deficit

| Option | Seed weight | | Straw weight | | Weight straw/ weight grain | Element depletion by spring wheat grain, mg/plant | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | g/plant g, M±m | Deviation of control, % | g/plant g, M±m | Deviation of control, % | | Nitrogen | Phosphorus | Potassium | Calcium | magnesium |
| Soil moisture 60–70% of full water capacity | | | | | | | | | | |
| Water (control) | 0.55 | 100 | 3.64 | 100 | 6.6 | 11.14 | 5.08 | 5.32 | 6.50 | 4.80 |
| Threonine solution | 0.64* | 116* | 3.25 | 89 | 5.1 | 20.48* | 7.16* | 5.76 | 6.72 | 4.80 |
| Nanocomposition solution based on $C_{60}$-threonine | 0.68* | 124* | 2.96* | 81* | 4.4* | 17.34* | 5.84* | 4.96 | 6.46 | 3.54* |
| Soil moisture deficit (soil moisture 25–30% of full water capacity) | | | | | | | | | | |
| Water (control) | 0.34* | 62* | 2.76* | 76* | 8.1* | 8.02* | 2.34* | 2.32* | 1.56* | 0.78* |
| Threonine solution | 0.59 | 107 | 2.80* | 77* | 4.8* | 14.76* | 3.42* | 3.96* | 2.40* | 1.98* |
| Nanocomposition solution based on $C_{60}$-threonine | 1.08* | 196* | 2.38* | 65* | 2.2* | 30.88* | 9.62* | 6.38* | 4.64* | 2.82* |

Note. * — value is definitely different from the control at 5% significance level.

by reproductive organs from non-productive vegetative part of plants which in total determines increase in plant resistance to soil moisture deficit, deficit of content of some trace elements which is shown by zinc and manganese, as well as to other stressors due to lower energy and resource consumption by plants during adaptation to stresses.

The revealed positive effect of synthesized water-soluble derivatives of fullerene $C_{60}$ and nanocompositions thereof in certain concentrations on production process in plants, their resistance to oxidative stress, high efficiency of these substances in small concentrations, and, respectively, low cost value of their application, and environmental friendliness are indicative of good perspectives of further study of effects of these substances on soil and vegetation system in order to develop and use high-efficient nanopreparations on their basis in crop production.

## Funding

The research was supported financially by the Agrophysical Research Institute using the funds of the program of fundamental scientific research in national academies of sciences for 2013–2020 stage № 0667-2019-0013 in terms of provision of vegetation and field experiments, with partial financial support by the Russian Foundation for Basic Research № 19-016-00003 in terms of evaluation of effect of fullerenol composition with manganese or zinc on resistance of cucumber plants, and the Russian Scientific Foundation № 22-26-00267 in terms study of the effect tris-malonate fullerene $C_{60}$ compositions with thionine complex on physiological condition of plants.

## Conflict of interests

The authors declare that they have no conflict of interest.

## References

[1] G.G. Panova, K.N. Semenov, O.A. Shilova, Yu.V. Khomiakov, L.M. Anikina, N.A. Tcharykov, A.M. Artemieva, E.V. Kanash, T.V. Khamov, O.R. Udalova. Agrofizika, **4**, 37–48 (2015).(in Russian)

[2] D. Deryabin, O. Davydova, Z. Yankina, A. Vasilchenko, S. Miroshnikov, A. Kornev, A. Ivanchikhina, P. Troshin. J. Nanomater., **9**, 907435 (2014). http://dx.doi.org/10.1155/2014/907435

[3] T. Baati, F. Bourasset, N. Gharbi, L. Njim, M. Abderrabba, A. Kerkeni, H. Szwarc, F. Moussa. Biomaterials, **33**, 4936–4946 (2012). DOI: 10.1016/j.biomaterials.2012.03.036

[4] J.J. Yin, F. Lao, J. Vehg, P.P. Fu, Y.L. Zhao, G. Xing, X. Gao, B. Sun, P.C. Wang, C. Chen, X.-J. Liang. Mol. Pharmacol., **74**, 1132–1140 (2008). DOI: 10.1124/mol.108.048348

[5] F. Beuerle, R. Lebovitz, A. Hirsch. Carbon Mater. Chem. Phys., *1*, 51–78 (2008).

[6] J.J. Yin, F. Lao, P.P. Fu, W.G. Wamer, Y. Zhao, P.C. Wang, Y. Qiu, B. Sun, G. Xing, J. Dong. X.-J. Liang. C. Chen. Biomaterials, **30** (4), 611–621 (2009). DOI: 10.1016/j.biomaterials.2008.09.061

[7] S.V. Shirinkin, M.I. Tchurnosov, G.V. Andrievsky, L.V. Vasil'chenko. Klinicheskaya meditsina, **5**, 56–58 (2009).(in Russian)

[8] L.V. Gerasimova, N.A. Charykov, K.N. Semenov, V.A. Keskinov, A.V. Kurilenko, Zh.K. Shaimardanov, B.K. Shaimardanova, N.A. Kulenova, D.G. Letenko, A. Kanbar. Nanosystems: Phys., Chem., Mathem., **12** (3), 346−362 (2021). DOI: 10.17586/2220-8054-2021-12-3-346-362

[9] Q. Zhao, Y. Li, J. Xu, R. Liu. W. Li. Int. J. Radiat. Biol., **81** (2), 169−175 (2005). DOI: 10.1080/09553000400029536

[10] F. Langa, J.-F. Nierengarten. *Fullerenes: Principles and Applications* (Royal Society of Chemistry, 2007)

[11] S. Trajkovic, S. Dobric, V. Jacevi, V. Dragojevic-Simi, Z. Milovanovic, A. Dordevi. Colloid. Surface B, **58** (1), 39−43 (2007). DOI: 10.1016/j.colsurfb.2007.01.005

[12] N.N. Shershakova, S.M. Andreev, E.N. Baraboshkina, D.D. Shabanova, E.A. Makarova, M.R. Khaitov. Immunologiya, **37** (6), (2016).(in Russian) DOI: 10.18821/0206-4952-2016-37-6-329-331

[13] S. Lin, J. Reppert, Q. Hu, J.S. Hudson, M.L. Reid, T.A. Ratnikova, A.M. Rao, H. Luo, P.C. Ke. Small, **5**, 1128−1132 (2009). DOI: 10.1002/smll.200801556

[14] R. Avanasi, W.A. Jackson, B. Sherwin, J.F. Mudge, T.A. Anderson. Environ. Sci. Technol., **48** (5), 2792−2797 (2014). DOI: 10.1021/es405306w

[15] C. Liang, H. Xiao, Z. Hu, X. Zhang, J. Hu. Environ. Pollut., **235**, 330−338 (2018). https://doi.org/10.1016/j.envpol.2017.12.062

[16] R. Chen, T.A. Ratnikova, M.B. Stone, S. Lin, M. Lard, G. Huang, J.S. Hudson, P.C. Ke. Small, **6**, 612−617 (2010). DOI: 10.1002/smll.200901911

[17] J. Gao, Y. Wang, K.M. Folta, V. Krishna, W. Bai, P. Indeglia, A. Georgieva, H. Nakamura, B. Koopman, B. Moudgi. PLoS ONE, **6** (5), e19976 (2011). DOI: 10.1371/journal.pone.0019976

[18] C. Kole, P. Kole, K.M. Randunu, P. Choudhary, R. Podila, P.C. Ke, A.M. Rao, R.K. Marcus. BMC Biotechnol., **13**, 37−58 (2013). DOI: 10.1186/1472-6750-13-37

[19] S. Samadi, B.A. Lajayer, E. Moghiseh, S. Rodríguez-Couto. Environ. Technol. Innov., **21**, 101323 (2021). DOI.org/10.1016/j.eti.2020.101323

[20] T. Kovac, T. Marcek, B. Šarkanj, I. Borišev, M. Ižakovic, K. Jukic, A. Loncaric, T. Krska, M. Sulyok, R. Krska. J. Fungi., **7**, 236 (2021). https://doi.org/10.3390/jof7030236

[21] G.G. Panova, I.N. Ktitorova, O.V. Skobeleva, N.G. Sinjavina, N.A. Charykov, K.N. Semenov. Plant Growth Regul., **79**, 309−317 (2016). DOI: 10.1007/s10725-015-0135-x

[22] F. Tai, Sh. Wang, B. Liang, Y. Li, J. Wu, Ch. Fan, X. Hu, H. Wang, R. He, W. Wang. J. Nanobiotechnol., **20**, 15 (2022). https://doi.org/10.1186/s12951-021-01222-7

[23] I. Andreev, A. Petrukhina, A. Garmanova, A. Babakhin, S. Andreev, V. Romanova, P. Troshin, O. Troshina, L. DuBuske. Fuller. Nanotub. Car. N., **16**, 89−102 (2008). DOI: 10.1080/15363830701885831

[24] C. Wang, H. Zhang, L Ruan, L. Chen, H. Li, X.-L. Chang, X. Zhang, S.-T. Yang. Environ. Sci. Nano., **4**, 1−7 (2016). DOI: 10.1039/C5EN00276A

[25] K.N. Semenov, A.A. Meshcheriakov, N.A. Charykov, M.E. Dmitrenko, V.A. Keskinov, I.V. Murin, G.G. Panova, V.V. Sharoyko, E.V. Kanash, Y.V. Khomyakov. RSC Advances, **7** (25), 15189−15200 (2017). DOI: 10.1039/C6RA26621E

[26] G.G. Panova, E.V. Kanash, Y.V. Khomyakov, A.M. Shpanev, E.B. Serebryakov, K.N. Semenov, O.S. Shemchuk, E.V. Andrusenko, N.E. Podolsky, V.V. Sharoyko, N.A. Charykov, L.L. Dulneva. J. Nanomater., 2306518 (2019). https://doi.org/10.1155/2019/2306518

[27] N.P. Bityutskii, K.L. Yakkonen, K.A. Lukina, K.N. Semenov, G.G. Panova. J. Plant Growth Regul., **40**, 1017−1031 (2020). https://doi.org/10.1007/s00344-020-10160-x

[28] G.G. Panova, A.S. Zhuravleva, Y.V. Khomyakov, V.E. Vertebnyi, S.V. Ageev, V.V. Sharoyko, K.N. Semenov, A.V. Petrov, N.E. Podolsky, E.I. Morozova. J. Mol. Struct., **1235**, 130163 (2021). DOI: 10.1016/j.molstruc.2021.130163

[29] *Sposob polucheniya smesi fullerenolov:* pat. 2495821 Ros. Federatsiya (in Russian). Semenov K.N., Tcharykov N.A., Namazbaev V.I., Keskinov V.A.; № 2010122963/05; zayavl. 04.06.2010; opubl. 20.10.2013, Byul. № 29.

[30] *Sposob polucheniya fullerenolov:* pat. 2481267 Ros. Federatsiya (in Russian). Semenov K.N., Tcharykov N.A., Keskinov V.A.; Keskinova M.V., Saf'yannikov N.M., Shubina V.A.; № 2011106276/05; zayavl. 11.02.2011; opubl. 10.05.2013, Byul. № 13.

[31] A.S. Vasilchenko, A.N. Smirnov, S.K. Zavriev, E.V. Grishin, A.V. Vasilchenko, E.A. Rogozhin. Int. J. Pept. Res. Ther., **23**, 171−180 (2017). https://doi.org/10.1007/s10989-016-9549-1

[32] G.G. Panova, K.N. Semenov,, O.A. Shilova, D.L. Kornyukhin, A.M. Shpanev, L.M. Anikina, T.V.Khamova, A.M. Artemieva, E.V. Kanash, N.A. Tcharykov, O.R. Udalova, A.S.Galushko, A.S.Zhuravleva, P.S. Filippova, D.V. Kudriavtsev, S.Yu. Blokhina. Agrofizika, **3**, 48−58 (2018).(in Russian) DOI: 10.25695/AGRPH.2018.03.09

[33] A.I. Ermakov, V.V. Arasimovich, M.N. Smirnova-Ikonnikova, N.P. Yarosh, G.A. Lukovnikova. *Metody biokhimicheskogo issledovaniya rasteniy* Pod red. A.I. Ermakova (Agropromizdat, Leningradskoye otd., L.., 1987), 430 p. (in Russian)

[34] E.M. Martinez, J.J. Cancela, T.S. Cuesta, X.X. Neira. Span J. Agric. Res., **9** (1), 313−328 (2011).

[35] A.I. Netrusov, M.A. Egorova, L.M. Zakharchuk, N.N. Kolotilova, I.B. Kotova, E.V. Semenova, N.Yu. Tatarinova, N.V. Ugol'kova, E.A. Tsavkelova, A.F. Bobkova, A.G. Bogdanov, I.V. Danilova, T.Yu.Dinarieva,V.V. Zinchenko, A.D. Ismailov, A.V. Kurvkov, V.N. Maksimov, E.S. Mil'ko, E.P. Nikitina, E.P. Ryzhkova, A.M. Semenov, D.V. Khomiakova, T.A. Tchardantseva, T.G. Yudina. *Praktikum po mikrobiologii* (Akademiya, M., 2005), 608 p. (in Russian)

[36] *Metodicheskiye ukazaniya po opredeleniyu tyazhelykh metallov v pochvakh s/h ugodiy i produktsii rastenievodstva* (TsINAO, 1992), http://docs.cntd.ru/document/1200078918

[37] *Rukovodstvo po metodam analiza kachestva i bezopasnosti pishchevykh productov,* pod red. prof. chl.-kor. MAI I.M. Skurikhina, akad. V.A. Tutel'yana (Brandes: Meditsina, 1998), 341 p (In Russian).

[38] T.A. Bankina, M.Yu. Petrov, T.M. Petrova, M.P. Bankin *Khromatografiya v agroekologii* (NII Khimii SPbGU, SPb., 2002), 580 p. (in Russian)

[39] A.C. Purvid, R.L. Shewfeld, J.W. Gegogeine. Physiol. Plantarum, **94**, 743−749 (1995). DOI: 10.1111/j.1399-3054.1995.tb00993.x

[40] A.C. Lukatkin. Fiziologiya rasteniy, **49**, 697−702 (2002). (in Russian)

[41] Kh.N. Pochinok. *Metody biokhimicheskogo analiza rasteniy* (Naukova Dumka, Kiev, 1976), 334 p. (in Russian)

[42] *Metodicheskiye ukazaniya po opredeleniyu nitratov i niriton v produktsii rastenievodstva MU № 5048-89.* (Ministerstvo zdravookhraneniya CCCR, Rosagroprom SSSR, Moskva, 1989), 52 p. (in Russian)

[43] E. Goiman. *Infektsionnye bolezni rasteniy* (Il., M., 1954), 610 p. (in Russian)

[44] R. Biczak, A. Telesiński, B. Pawłowska. Plant Physiol. Bioch., **107**, 248–256 (2016). DOI: 10.1016/j.plaphy.2016.05.016

[45] B. Zhang, X. Li, D. Chen, J. Wang. Protoplasma, **250**, 103–110 (2013).

[46] R. Jbir-Koubaa, S. Charfeddine, W. Ellouz, M.N. Saidi, N. Drira, R. Gargouri-Bouzid,, O. Nouri-Ellouz. Plant Cell Tiss. Organ. Cult., **120** (3), 933–947 (2015).
DOI: 10.1007/s11240-014-0648-4

[47] R.A. Azevedo, M. Lancien, P.J. Lea. Amino Acids, **30**, 143–162 (2006). DOI: 10.1007/s00726-005-0245-2