Effect of “low doses” of multiwall carbon nanotubes when interacting with white mustard seeds and sprouts

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Abstract. The paper considers the influence of the sizes of carbon nanotubes (CNT) on the effect of “low doses” (LD) by changing the morphological, physiological and biochemical parameters of seeds and sprouts of white mustard. In the first series of experiments, 20 nm nanoparticles were studied. The second series considered 40-60 nm nanoparticles at concentrations of 0.01 to 1,000 g per ton of seeds. For CNT, the effect of “low doses” with particle sizes of 40-60 nm had its own characteristics. The germination energy and viability were inhibited or did not exceed the control at all concentrations studied with the exception of a concentration of 0.01 g / t when the root length increased by 18.5%. But the analysis of the mass of 7-day-old white mustard sprouts showed that the mass of the experimental sprouts exceeded the control in all cases. The change in the mass of 7-day-old mustard roots was “dome-shaped” and the maximum fell on a CNT dose of 5.0 g / t (+ 42.3% above the control). Carbon nanoparticles up to 20 nm in size showed higher activity in almost all parameters. Energy was higher than the control up to 10 g / t. Concentrations from 100 to 1,000 g / t had a depressing effect on this parameter. Biochemical studies confirmed the results of morphophysiological studies.

1. Introduction

In the last decade, the attention of researchers has been attracted by the problem of the biological effect of preparations in low (LD) and ultra-low (ULD) doses. A small exposure causes biological effects comparable or even stronger than those observed with higher doses of the substance. At present, no single mechanism for the biological action of physical and chemical factors, as well as preparations in LD and ULD, has been proposed. In addition, the proposed mechanisms of action of biologically active substances in ULD are based on the assumption of passive diffusion, the result of which is the interaction of single ligand molecules and cell receptors [1–3].

The nature of the biological effects caused by various factors in LD indicates that the cause of this phenomenon is the processes associated with the action of a certain regulatory signal in biological systems.

Gusev A.A. and other scientists revealed that multiwall carbon nanotubes (MWCNTs), when interacting with plant sprouts, are concentrated in the stomata region, which indicates the important role of transpiration in the bioaccumulation of this type of nanomaterials. The ability of MWCNTs to penetrate and bioaccumulate in plants indicates a high potential hazard. Nanoparticles suppressed the...
processes of seed germination of *O. arenaria* seeds by 21 - 39 %. They had some multidirectional effect on the growth of stems (from –26 to +21%) and positive (up to +76%) on root growth. The increased growth of sainfoin roots is probably due to the hypothesis of an increase in water intake due to "piercing" of the seeds skin by MWCNTs. The effect of MWCNTs on plants is not linearly related to concentration and differs from the action of carbon of another allotropic modification (soot). An analysis of the effect of MWCNTs on the antioxidant system of sainfoin sprouts showed a decrease in catalase activity at all concentrations, with the exception of the MWCNT 1 g / l group, where an increase in the studied parameter was noted. The lowest enzyme activity (1.5 - 2 times lower than the control value) was observed at the minimum and maximum concentrations of MWCNTs. The peroxidase activity in sainfoin sprouts under the influence of MWNTs did not significantly differ from the control values. A study of the effect of MWCNTs on the activity of polyphenol oxidase showed its decrease, with the exception of the group “MWCNTs 10 mg / l”, where the maximum value was recorded that exceeded the control 1.5 times.

The minimum values of the content of chlorophylls a, b and carotenoids were recorded in MWCNTs 10 mg / l and MWCNTs 10 g / l, the maximum value was found in MWCNT 1 mg / l group. In other cases, MWNTs had an inhibitory effect on the photosynthetic sainfoin system, which corresponded to the noted changes in the germination rate and activity of antioxidant enzymes. It was established that the presence of MWCNTs in the medium of seed germination had a significant effect on the concentration of phytohormones in *O. arearia* sprouts. With an increase in the concentration of MWCNTs in the test medium, the concentration of indolylacetic acid increases. In this case, the concentration of cytokinins, on the contrary, decreases [4–6].

We have long studied the biological activity of nanoparticles of various nature, depending on the method of preparation, particle sizes and their concentrations [7–10]. The results of many years of research show that at low concentrations, nanoparticles, including carbon ones, can exhibit a biological effect [11–15]. Therefore, the study of the effect of "low doses" of nanoparticles is relevant and of scientific interest.

This study examined the effect of carbon nanomaterial sizes on vital and morpho-physiological parameters of white mustard seeds. For research, multiwall carbon nanotubes (MWCNTs) with a particle size of 20 nm and 40-60 nm were selected.

2. Scheme and research methods

Multiwall carbon nanotubes were synthesized by gas-phase chemical deposition. Their production was mastered by NanoTechCenter LLC in Tambov. Using methods of transmission and scanning electron microscopy, it was found that the diameter of the nanotubes was 20 nm, the length was 5 - 10 microns and the distance between the layers of carbon atoms in nanotubes was about 0.25 nm. Multiwall carbon nanotubes form agglomerates ranging in size from 1 to 1,000 microns with the structure of tangled CNT beams.

![Figure 1. TEM analysis of MWCNTs using electron diffraction: a) Ultra-thin section of a sample of MWCNTs. b) Slice region analyzed by electron diffraction. c) Diffraction pattern of this site.](image-url)
MWCNT suspensions were prepared on the basis of distilled water. Samples (from 1 mg to 10 g in 10-step increments) were weighed using a ViBRA HT analytical balance (Shinko Denshi, Japan, accuracy ± 0.0001 g), poured into a pre-prepared container with 1 liter of water and mixed with a glass rod for 20 seconds. Then they were dispersed using ultrasound for 5 minutes, power - 300 W, frequency - 23,740 kHz according to the developed sample preparation technique. IKA MagicLab MK homogenizer-dispersant (IKA Werke, Germany) was used as a mechanical dispersant. The speed of the homogenizer-dispersant in the manufacture of carbon nanoparticle suspensions using mechanical stresses was 20,000 rpm, pressure 2.5 bar and processing time 5 and 10 minutes. Distilled water for control experiments was treated in a similar way without adding any nanoparticles (NPs).

The studies were conducted at the Center for Nanotechnology and Nanomaterials for Agroindustrial Complex at Ryazan State Agrotechnological University named after P.A. Kostychev. Seeds were laid in Petri dishes with 50 seeds each in 4-fold repetition. Then they were placed in a thermostat, where they germinated at a constant t = 23° C. Germination energy and viability were determined in accordance with GOST 12038-84. The length of the sprouts and roots was calculated using a ruler for each plant with sprouts or roots and the mass of sprouts was measured on a Ohaus digital analytical balance.

The activity of peroxidase and superoxide dismutase enzymes in sprouts and roots of white mustard was determined using an SF-2000 spectrophotometer (ZAO OKB SPECTR, Russia).

Statistical data processing was carried out using Statistica 6 electronic software package. Control seeds were soaked in distilled water, and experimental seeds were soaked in a solution of CNT preparations of a certain concentration (Table 1).

| #  | Variant  | Concentrations, g / ton of seeds |
|----|----------|----------------------------------|
| 1. | Control  | seeds are soaked for 30 minutes in distilled water |
| 2. | CNT, 20 nm | 0.01; 0.05; 0.1; 0.5; 1.0; 5.0; 10.0; 100.0; 500.0; 1,000.0 |
| 3. | CNT, 40-60 nm | 0.01; 0.05; 0.1; 0.5; 1.0; 5.0; 10.0; 100.0; 500.0; 1,000.0 |

To search for the dose-effect relationship, it was decided to determine parameters that clearly demonstrate a stimulating, inhibitory effect or its absence - germination energy, laboratory viability, length and weight of 7-day-old mustard sprouts in laboratory conditions, as well as the content of enzymes in roots and sprouts of white mustard.

3. Results and discussion

Table 2 and Figures 2-3 show the interaction results of CNT with a particle size of 40-60 nm in various concentrations and seeds of white mustard.

The germination energy (day 3 of the experiment) in almost all concentration variants was significantly lower than the control, with the exception of 0.5 g / t CNT concentration. This parameter was equal to the control value. When analyzing the viability (day 7 of the experiment) of white mustard seeds (Figure 2), one can also observe one “peak” of the parameter - but already at a concentration of 0.05 g / t of seeds.

The length of the mustard sprout, measured on the 3rd day, was significantly lower than the control in all cases (2-3 times), but jumps were nevertheless observed at doses of 5.0 and 500.0 g / t of seeds. The root length on the 3rd day of the experiment exceeded the control only at a dose of 0.01 g / t (+ 18.5%), in other cases it was 80-90% lower than the control.

Similarly to the length, the mass of 3 day old sprouts and roots also changed - it was lower than the control value in almost all parameters.

A different picture is observed when analyzing the mass of 7-day-old sprouts of white mustard (Table 2). The mass of 1 sprout exceeds the control in all variants, but the maximum value is observed at a CNT concentration of 100.0 and 1,000.0 g / t of seeds (+ 170.5% and + 176.7%, respectively,
above the control). The change in mass of the 7-day-old mustard roots is “domed” and the maximum is at a dose of CNT of 5.0 g / t (+ 42.3% above the control).

Table 3 and figures 4, 5, 6 present the results of the interaction of multiwall carbon nanotubes with a particle size of 20 nm with white mustard seeds and sprouts.

Carbon nanoparticles up to 20 nm in size show higher activity in almost all parameters. Thus, the germination energy is higher than the control for all variants up to 10 g / t, with the peak value falling at a dose of 1.0 g / t (+ 12.6%). Concentrations from 100 to 1,000 g / t had a depressing effect on this parameter. An increase in viability of white mustard seeds relative to the control was also observed in all cases, except for the maximum doses - 500.0 and 1,000.0 g / t. But the best result was observed at two concentrations - 0.1 g / t (+ 11.6%) and 1.0 g / t (+ 11.4% compared with the control).

The length of 3-day-old sprouts reached its maximum at a concentration range of 5.0 - 100.0 g / t (+ 93.6%), and the length of 3-day-old roots was maximum at a CNT of 0.5 g / t and exceeded the control by 175.7%.

**Table 2.** Vital and morpho-physiological parameters of white mustard sprouts when interacting with CNT with a particle size of 40-60 nm

| Variant | Germination energy, % | Mass of aboveground sprout, g | Mass of underground sprout, g | Mass of aboveground sprout, g | Mass of underground sprout, g |
|---------|-----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Control | 76.6±1.4              | 0.0157±0.0004                 | 0.0063±0.0001                 | 0.0129±0.0007                 | 0.0052±0.0002                 |
| CNT 0.01| 66.5±1.1              | 0.0121±0.0003                 | 0.0079±0.0002                 | 0.0310±0.0006                 | 0.0073±0.0001                 |
| CNT 0.05| 66.7±1.3              | 0.0134±0.0003                 | 0.0024±0.0001                 | 0.0295±0.0005                 | 0.0069±0.0003                 |
| CNT 0.1 | 65.6±1.3              | 0.0115±0.0004                 | 0.0025±0.0003                 | 0.0296±0.0004                 | 0.0071±0.0004                 |
| CNT 0.5 | 76.6±1.5              | 0.0123±0.0003                 | 0.0027±0.0001                 | 0.0282±0.0006                 | 0.0072±0.0001                 |
| CNT 1.0 | 67.5±1.3              | 0.0136±0.0005                 | 0.0034±0.0005                 | 0.0277±0.0003                 | 0.0073±0.0006                 |
| CNT 5.0 | 68.9±1.4              | 0.0144±0.0006                 | 0.0029±0.0001                 | 0.0295±0.0006                 | 0.0074±0.0001                 |
| CNT 10.0| 62.4±1.2              | 0.0112±0.0003                 | 0.0024±0.0003                 | 0.0327±0.0007                 | 0.0073±0.0005                 |
| CNT 100.0| 46.0±0.4            | 0.0129±0.0004                 | 0.0030±0.0001                 | 0.0349±0.0005                 | 0.0065±0.0002                 |
| CNT 500.0| 42.3±0.3            | 0.0123±0.0003                 | 0.0016±0.0002                 | 0.0326±0.0007                 | 0.0066±0.0002                 |
| CNT 1,000.0| 39.0±0.2         | 0.0018±0.0002                 | 0.0010±0.0003                 | 0.0357±0.0006                 | 0.0038±0.0001                 |
| LSD<sub>05</sub> | 2.5 | 0.0019 | 0.0014 | 0.0042 | 0.0011 |

**Figure 2.** Germination of white mustard seeds treated with CNT 40-60 nm
When analyzing the mass of sprouts and roots of white mustard (Table 3) during interaction with carbon nanotubes, the following regularities can be distinguished. The mass of 3-day-old sprouts and roots changes "dome-shaped", reaching a maximum value at CNT 10.0 (+94% above the control) and CNT 5.0 (+5.1%), respectively.

With a longer contact between CNT and mustard, the mass of the sprouts changes "stepwise" - 2 peaks are visible on the graph — at 0.5 and 10.0 g/t. The same jumps are observed when analyzing the mass of 7-day-old roots, the graph peaks are at doses of 1.0 and 10.0 g/t.

Table 3. Vital and morpho-physiological parameters of white mustard sprouts when interacting with CNT with a particle size of 20 nm

| Variant | Germination energy, % | 3-day-old sprouts | 7-day-old sprouts |
|---------|---------------------|------------------|------------------|
|         |                     | Mass of aboveground sprout, g | Mass of underground sprout, g | Mass of aboveground sprout, g | Mass of underground sprout, g |
| Control | 82.0±2.8            | 0.0218±0.0008     | 0.0203±0.0006     | 0.0549±0.0015     | 0.0385±0.0008     |
| CNT 0.01| 94.4±1.7            | 0.0373±0.0007     | 0.0274±0.0005     | 0.0871±0.0017     | 0.0440±0.0009     |
| CNT 0.05| 93.8±1.5            | 0.0406±0.0005     | 0.0302±0.0007     | 0.0974±0.0019     | 0.0470±0.0009     |
| CNT 0.1 | 94.5±1.6            | 0.0407±0.0006     | 0.0302±0.0002     | 0.1090±0.0022     | 0.0500±0.0010     |
| CNT 0.5 | 94.4±1.2            | 0.0407±0.0004     | 0.0301±0.0004     | 0.1216±0.0024     | 0.0655±0.0013     |
| CNT 1.0 | 94.6±1.3            | 0.0408±0.0006     | 0.0306±0.0005     | 0.1126±0.0023     | 0.0702±0.0014     |
| CNT 5.0 | 94.5±1.5            | 0.0413±0.0008     | 0.0308±0.0009     | 0.1199±0.0024     | 0.0693±0.0014     |
| CNT 10.0| 88.6±1.4            | 0.0423±0.0009     | 0.0313±0.0008     | 0.1590±0.0032     | 0.0715±0.0014     |
| CNT 100.0| 81.6±1.6           | 0.0274±0.0007     | 0.0273±0.0005     | 0.1058±0.0021     | 0.0513±0.0010     |
| CNT 500.0| 18.0±0.5           | 0.0117±0.0006     | 0.0180±0.0007     | 0.0525±0.0012     | 0.0312±0.0006     |
| CNT 1,000.0| 7.0±0.2           | 0.0109±0.0005     | 0.0143±0.0006     | 0.0454±0.0010     | 0.0108±0.0002     |
| LSD_{05} | 1.8                | 0.0032            | 0.0027            | 0.0035            | 0.0018            |
Figure 4. Viability of white mustard seeds treated with CNT 20 nm

Figure 5. The length of white mustard sprouts treated with CNT 20 nm

The obtained results are consistent with the results of other scientists. In [16,17], it was shown that the studied CNT accelerate the growth of all plant organs — roots, sprouts, and stalks of tomatoes, although they have similar sizes, but a different structure. The team of authors [18-20] sticks to the point that CNT and other nanoparticles contribute to the formation of additional “channels” in plants through which water and nutrients dissolved in it efficiently move into the plant. With this phenomenon, researchers attribute the intensification of biosynthesis in plant cells under the influence of CNT.

At the next stage, enzymes peroxidase and superoxide dismutase [21] were determined in sprouts and roots of white mustard, the seeds of which were treated with CNT particle sizes of 40-60 nm (Table 4) and 20 nm (Figures 6, 7).

The effect of “low doses” of carbon nanotubes with a particle size of 40-60 nm on the activity of enzymes in mustard sprouts showed that the activity of peroxidase in mustard roots was higher than the control at low concentrations (from 0.01 to 10.0 g / t), and decreased significantly at high ones of 100 - 1,000 g / t (-8.1-22.0%). In mustard sprouts, a positive effect of low concentrations was observed in all experimental variants, with the exception of the maximum concentration at which activity decreased by 39.3% below the control.
The activity of the superoxide dismutase in mustard sprouts when interacting with CNT was higher than the control in almost all variants, both in the roots and in the sprouts, reaching a peak at 100.0 g / t. A decrease in this parameter was observed only at a maximum concentration of 1,000 g / t of seeds.

**Table 4.** The activity of peroxidase (in unit opt. dens. / g raw tissue • sec) and superoxide dismutase (in c.u. / g raw tissue) in roots and sprouts of mustard when interacting with CNT (40-60 nm)

| Variant | PEROXIDASE |            |            |            |            |            |            | SUPEROXIDE DISMUTASE |            |            |            |
|---------|------------|------------|------------|------------|------------|------------|------------|----------------------|------------|------------|------------|
|         | Roots      | Sprouts    | Roots      | Sprouts    | Roots      | Sprouts    |            |                      |            |            |            |
|         | abs. value | % to control | abs. value | % to control | abs. value | % to control | abs. value | % to control | abs. value | % to control | abs. value | % to control |
| Control | 6.95       | -          | 3.51       | -          | 51.27      | -          | 78.06      | -                    | 82.62      | +5.8       | 92.26      | +16.4       |
| CNT – 0.01 | 7.04     | +1.3       | 3.89       | +10.8      | 53.82      | +4.9       | 82.62      | +5.8                  | 92.26      | +25.9      | 92.26      | +25.9       |
| CNT – 0.10 | 7.43      | +6.9       | 3.96       | +12.8      | 52.23      | +1.9       | 98.24      | +25.9                | 92.26      | +25.9      | 92.26      | +25.9       |
| CNT – 1.00 | 8.56      | +23.1      | 4.16       | +18.5      | 54.15      | +5.6       | 83.05      | +6.4                  | 96.43      | +23.5      | 92.26      | +23.5       |
| CNT – 10 | 7.34       | +5.6       | 4.86       | +38.5      | 62.68      | +22.3      | 92.26      | +18.2                | 92.26      | +18.2      | 92.26      | +18.2       |
| CNT – 100 | 6.39      | -8.1       | 3.62       | -3.1       | 74.55      | +45.4      | 96.43      | +23.5                | 92.26      | +23.5      | 92.26      | +23.5       |
| CNT – 1,000 | 5.42     | -22.0      | 2.13       | -39.3      | 48.81      | -4.8       | 69.15      | -11.4                | 92.26      | -18.2      | 92.26      | -18.2       |

**Figure 6.** Peroxidase activity in experimental samples of mustard (roots and sprouts) when interacting with CNT 20 nm

Peroxidase reacts differently to the presence of carbon nanotubes (CNTs), which are at different concentrations in a gel-like (polysaccharide) culture media. The peroxidase activity at the location is different: roots, sprouts at the same concentration of nanoparticles. However, the pattern of changes in the activity of peroxidase for mustard is approximately the same.

With an increase in the content of carbon nanotubes, an increase in the enzyme activity in mustard roots is observed from 2.6% (CNT - 0.01 g / t) to 18.0% (with a CNT content of 10 g / t). At a higher content of nanotubes, the peroxidase concentration decreases to 6.2% (CNT - 100 g / t) and 23.2% (CNT - 100 g / t) (Figure 6).

The activity of peroxidase in sprouts at low concentrations of carbon nanotubes decreases from 13.1% above the control for 0.01 g / t to 8.5% (1.0 g / t). Then the activity decreases more significantly -3.2% (10 g / t), -8.0% (100 g / t), -14.0% relative to the control (for a concentration of 1,000 g / t).
Figure 7. Superoxide dismutase activity in experimental samples of mustard (roots and sprouts) when interacting with CNT 20 nm

The amount of superoxide dismutase enzyme in the roots of mustard prototypes gradually increases to 10.2% (CNT-1.0 g / t) with an increase in the content of carbon nanotubes, then increases sharply, reaching a maximum of + 78.6% (CNT-100 g / t) and with a CNT content - 1.000 g / t is reduced to 24.3%.

The activity of superoxide dismutase in the mustard sprouts in solutions of low concentration carbon nanotubes decreases, but it increases with a content of CNT -1.0 g / t from -2.7% to + 1.3%, CNT -100.0 g / t, and with a concentration of CNT-1,000 g / t it drops sharply to - 56.7%. It should be noted that the activity of superoxide dismutase in sprouts is several times higher than in mustard roots.

4. Conclusion
Based on the above results and summarizing the data, one can draw the following conclusions:

1. For carbon nanotubes, the effect of "low doses" is most pronounced at a particle size of 20 nm. Thus, the germination energy is higher than the control up to 10 g / t, and the peak value falls to 1.0 g / t (+ 12.6%). Concentrations from 100 to 1,000 g / t had a depressing effect on this parameter. An increase in the viability of white mustard seeds relative to the control was also observed in all cases, except for the maximum doses - 500.0 and 1,000.0 g / t. The change in the mass of 7-day-old mustard roots is “dome-shaped” and the maximum falls on a CNT dose of 5.0 g / t (+ 42.3% above the control).

2. Biochemical studies confirmed the results of morpho-physiological studies, CNT with a particle size of 20 nm changed the activity of enzymes as follows: an increase in the activity of peroxidase in mustard roots was observed in the CNT concentration range of 0.01 - 10 g / t and a further increase in doses decreased the activity of the enzyme. The peak of peroxidase activity in sprouts was observed at CNT0.001 g / t (+ 13.1% higher than the control), then there was a decrease in the parameter. For superoxide dismutase, the peak was observed in mustard roots at CNT-100 g / t (+ 78.6%). This enzyme decreases in mustard sprouts at a low concentration. It should be noted that the activity of superoxide dismutase in sprouts is several times higher than in mustard roots.

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