характерно для наполненных полимерных систем. Связано это с тем, что, как было указано выше, перераспределение наночастиц в аморфных областях полукристаллического ПЭНП способствует блокированию проходных макроцепей, ответственных за удлинение образцов. Одновременно установлено, что термозола способствует улучшению текучести расплава наноккомпозитов. Обнаруженный эффект связан с тем, что в составе термозолы наряду с различными окислами металлов содержится графит, который, как известно, способствует улучшению текучести расплава.

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Factors affecting the biological activity and the mode of action of metal nanoparticles

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Abstract

Nanoparticles of iron, copper and cobalt metals enhance the morpho-physiological and biochemical parameters of plants, which makes it possible to use nanoparticles as growth stimulants with a prolonged effect. The chemical interaction of nanoparticles with a liquid medium is one of the determining factors in their biological activity. For metal nanoparticles up to 60 nm in size, accumulation is not proven, but their permeability to the cell takes place. In this case, the transmembrane potential changes - the energy increases, the activity of synthesis of enzymes and phytohormones, IAA in particular, also increases, and plant growth and development are enhanced. The observed biological effects are caused by nanoparticles of the same size with different physical-chemical properties. Therefore, the causes of these phenomena are processes associated with the action of a certain regulatory signal in biological systems. Changes in the structure of membranes in the presence of nanoparticles can lead to some change in the functional state of the cell and a change in the mode of action of the substance in a different concentration range.

Introduction

One of the characteristic properties of the metal nanoparticles' LD effect is observed - the oscillatory nature of the dose dependence of the magnitude of the observed parameters, as well as the presence of a "dead zone" (dose interval between two peaks of biological effects in which biological activity is not manifested). This is due to the wave nature of the propagation of spatial rearrangements of the permeability of membranes and supramolecular structures under the influence of the energy effect of nanoparticles, increasing the concentration of protons. This corresponds to the obtained results: with an increase in the concentration of nanoparticles (active substance), the morpho-physiological processes of plants and the activity of enzymes and phytohormones change [1-5]. The biological system being influenced by low doses does not obey the law of the acting masses and responds to the first, single doses of nanoparticles, and not to concentration in a stationary state. It is believed that the biological effects produced by nanoparticles are based on a phased change in the spatial organization of specialized supramolecular structures of the microenvironment of the cell and its cytoplasm (ECM, plasma membrane, cytoskeleton, nuclear matrix), for each of which the significance in the course of the most important biological processes has been identified and proven.

Low and ultra-low doses are those ones whose effectiveness cannot be explained with the help of traditional concepts. Therefore, the nanoparticles of trace elements themselves, which are characterized by quantum-mechanical properties, can be attributed to "low or ultra-low". Biological activity in its quantitative expression obeys the dependence of concentration - property. One of the manifestations of stresses, including nanoparticles, is the presence of an adaptive response, which is a universal response of cells to an irritant in "low" doses, and the acquisition of resistance to the damaging effects of NPs in a "large" dose. In turn, the biological activity of nanoparticles in different concentration ranges depends on the particle size. Nanoparticles up to 20 nm in size show higher activity at low concentrations. For example, when treating plant seeds before planting with metal nanoparticles up to 5 g/t (ton of seed sowing), for nanoparticles at 0.05 g/t relative to the control, the germination energy increases by 16-20%; germination by 14 -16%. The length of the aerial part is up to 60% and the length of the underground part is 128% that is much higher than that of particles with a higher size of 35-60 nm at the same concentration: only 10-12%, 8-14%, 37%, -40% and 18-25%, respectively. Theoretical and experimental studies of small particles show that particle size is an active variable that determines the state of the system. Smaller nanoparticles have a high interface. As a result, such nano-objects have high physical-chemical activity. The objects of study are the energy of seed germination and their morpho-physiological parameters. The results show that "low" doses are more effective, because organisms apparently do not have a system of protection against them and their signal stimulates the processes of seed germination to a greater extent. In the range of "low" doses per unit mass of grain, the influence effect is much higher than at "high" doses, but the effect of nanoparticles increases with increasing concentration to a certain limit. However, in practice it is dangerous to use small nanoparticles, since it is difficult to control their high activity at extremely low concentrations.

Nanoparticles 100 nm in size and above showed an effect on the studied parameters only on the 7th day of the experiment and at very high concentrations (100-1,000 g/t). Electron microscopic analysis with the help of transmission electron microscope "JEOL" "JEM-1400" [6] showed that after processing the plants with a colloidal solution of nanoparticles larger than 100 nm, they were concentrated in large quantities on the plant surface, probably due to their pronounced adhesive properties. In the course of further electron microscopic studies, it was possible to detect interstitial bioaccumulation of nanoparticles in 3 and 7-day-old sprouts. However, the additional elemental analysis did not show their reliable accumulation in plant tissues compared with the control. Therefore, possible negative effects can only be associated with the individual effects of nanoparticles, and not with the accumulation of heavy metals in plants and soil. For example, if an

elemental analysis of a sample of the homogenate of the roots and sprouts of plants of a group exposed to Cu nanoparticles above 100 nm determines the accumulation of small amounts of this element in the tissues and there are peaks characteristic of Cu in the diagrams (Figure 1), then their accumulation on the surface of cells of the aerial and underground parts were found (Figure 2). In the ratio of the content of the remaining elements, deviations from control values were not recorded.

Figure 1 - Elemental analysis of a sample of mustard sprout homogenate after 20 days of seed contact with suspensions of copper nanoparticles, size 100 nm, concentration 100 g / t

Figure 2 - Micrographs from a scanning microscope of the surface of the cells of the sprouts: a - control samples; b - sprouts after 20 days of mustard seeds contact with suspensions of copper nanoparticles

Particles sized 35-60 nm are more biologically safe and active. They do not accumulate either in plants or in soil [7-10]. For nanoparticles of iron, copper, cobalt obtained chemically, with particles sized 35-60 nm, the effect of "low doses" is most pronounced. At concentrations above 100 g / t, the dose-effect dependence ceased to manifest itself, but all parameters remained significantly higher than the control.

Metal nanoparticles exhibit almost the same dependence in essence, but different in magnitude. This can be attributed to the unity and difference of some of their physical-chemical characteristics. When characterizing nanoparticles, the most important ones were identified that affect their biocompatibility and, as a consequence, biological activity. Table 1 presents some characteristics of the studied nanoparticles.

For nanoparticle suspensions, a change in pH was proved depending on the composition and size of the nanoparticles. A direct correlation between the increase in the number of protons and the energy produced in the cells, which is used for seed germination, is suggested. This refers to an increase in the permeability of cell membranes, the activity of enzymes and phytohormones. In recent years, more and more works have appeared devoted to the study of intracellular pH and its role in cell activity. The activity of enzymes, the transport of substrates through membranes and the direction of enzymatic reactions depend on the pH

value in the cell [11,12]. The relationship between hydrogen ions and the energy produced in cells and used for germination is confirmed by recent studies in the field of enzymes including the one in the framework of this project.

Table 1

Characteristics of the studied nanoparticles

	Cu	Co	Fe	Zn
Particle size in one of the measurements, nm	34-63	28-46	41-44	30-66
Solubility in water wt. %	Insoluble	Insoluble	Insoluble	Insoluble
Solubility in biological fluids (physical solution) wt. %	<1	<1	Insoluble	Insoluble
Charge	+	+	+	+
Aggregation resistance	Low	Low	Low	Low
Hydrophobic nature	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic
Adhesion to plant surfaces	detected by electron microscopy	detected by electron microscopy	detected by electron microscopy	detected by electron microscopy

An increase in the content of hydrogen cations in a solution containing 100 g / t Fe occurred 4.4 times (particle size 35-60 nm) and that in a solution containing 10 g / t Fe was 13.5 times in comparison with double-distilled water. Therefore, at a lower concentration, the availability of surface iron atoms for interaction with water with the formation of protons is higher. For nanoparticles up to 20 nm in size at a concentration of 10 g / t Fe, the content of hydrogen cations increased only 15 times, and for 0.01 g / t it was 17 times. A similar dependence was also observed for cobalt and copper nanoparticles. Perhaps this is also their high biological activity at low concentrations.

When studying the activity of some enzymes, it was proved that the effect of “low doses” of metal nanoparticles sized 35-60 nm has common patterns for all crops but is quantitatively different. Thus, the activity of the peroxidase enzyme in roots of sprouts exceeds the control within the experiment up to 30% to 100 g / t at a maximum of 1.0 and 10.0 g / t (+ 16-21% higher than the control). A further increase in the concentration of NP leads either to decreased activity, or an increase of more than 30%, which indicates inhibition of plant growth and development. At the same time, a wave-like dose-response relationship is maintained. Some positive effect of low concentrations was observed in sprouts at 0.01; 0.1 and 1.0 g / t NP. Other concentrations had a negative result.

The activity of the superoxide dismutase enzyme in roots changed “dome-shaped”, reaching a peak at 100.0 g / t (+ 47.0% above the control). The activity of superoxide dismutase in sprouts at low concentrations of nanoparticles was lower than the control, and then increased. The maximum value was at 100.0 g / t and that was 40-220% higher than the control.

An analysis of the effect of “low doses” of nanoparticles sized up to 20 nm showed that the activity of the peroxidase enzyme in roots of sprouts exceeded the control for all experimental variants, at a maximum of 10.0 g / t (+ 49.9% higher than the control), which suggests inhibition development at a given concentration. The positive effect of low concentrations of cobalt NP was observed in sprouts at low doses of 0.01-10.0 g / t. At a maximum of 0.10 g / t it exceeded the control by 19.5% and large concentrations of 100-1,000 g / t gave a negative result. The activity of the superoxide dismutase enzyme both in the roots and in the sprouts when interacting with NPs of 20 nm was higher than the control in all cases, reaching a peak at 100.0 g / t of seeds. Under the action of metal nanoparticles, the activity of catalase decreased at low concentrations of nanoparticles. However, with an increase in the concentration, deviations from the control increased by 40-55% as compared with the control.

A comprehensive study of the effect of various concentrations and sizes of nanoparticles on the activity of three antioxidant enzymes (SOD, catalase and peroxidase) made it possible to establish that peroxidase activity at high morpho-physiological parameters was high and decreased in “dead zones” both at low and high concentrations of metal nanoparticles. The activity of SOD and catalase in the studied crops significantly decreased when maximum morpho-physiological parameters and sharply increased at concentrations above 100 g / t for nanoparticles with a size of 35-60 nm and above 10 g / t with a size of up to 20 nm. For nanoparticles with a size of 35-60 nm, the concentration limit is much higher than 100 g / t for stability and for sizes of up to 10 nm it was in the range of 10-100 g / t. As in the case of morpho-physiological parameters, the activity of enzymes corresponds to the normal development of plants to concentrations of 10 g / t with particle sizes of up to 20 nm and to 100 g / t with particle sizes of up to 35-60 nm. Then there is no growth stimulation.

At different stages of plant development and growth, the ratio of phytohormones changes [13-15] both in the sprouts and in the roots under the influence of various environmental factors. Such changes correct the direction and growth rate of plant organisms. Hormones model the entire process of plant development, can change its direction, exerting effects on enzymes that regulate the activity of other hormones. The effect of nanoparticles (NPs) of cobalt, iron, zinc and copper, phytohormones of cytokinins (CK), gibberellic (GA), abscisic (ABA), and indolylacetic (IAA) acids on white mustard, grown in a sandy substrate for a month, was studied.

In the concentration range of 0.001-10.0 g / t under the influence of metal nanoparticles, the content of IAA increases. Therefore, at these concentrations of nanoparticles, the energy efficiency of plant respiration increases and this leads to large changes in the activity of various enzymatic reactions. NPs should enhance the movement of nutrients and water, which is one of the reasons for the increased plant growth that was observed in previous experiments. The increase in the content of gibberellic, abscisic and indolylacetic acids is maintained up to a concentration of 10 g / t, and most intensively, with an increase in the concentration of NPs of 30-60 nm in size. Such conclusions are reliably characteristic of the action of nanoparticles of cobalt, copper, zinc and iron.

For nanoparticles of up to 20 nm in size, a similar dependence was generally observed, as in the case of particles with a size of 35-60 nm. At the same time, a higher activity of CK, GA and IAA was characteristic of control at lower concentrations of 0.01–1.0 g / t and these changes were higher than for nanoparticles with a size of 35-60 nm. It should be noted that the amount of abscisic acid practically did not change, there was no dose-effect dependence for this hormone. The highest IAA activity was observed in the range of 1-10 g / t of cobalt nanoparticles with a size of 35-60 nm and for sizes of up to 20 nm the activity shifted toward lower concentrations of 0.1-1.0 g / t. At these concentrations the activity of the remaining phytohormones increased with a decrease in the ABA content.

If we take into account that nanoparticles are a source of additional protons and information that cells receive from high-energy nanoparticles through membrane or cytoplasmic receptors, then these factors should lead to a change in hormonal status in cells and stimulate or suppress the activity of certain endogenous phytohormones. And the information depends on the size, composition and concentration of the nanoparticles. Biologically active nanoparticles, acting on the level of phytohormones, can affect the state of biomembranes and indirectly act as a regulator of protein synthesizing plant cells [16]. And this leads to the growth and development of living systems.

Conclusion

For metal nanoparticles of up to 60 nm in size, accumulation is not proven, but their permeability to the cell takes place. The walls of plants are semi-permeable, the pore diameter in the cell walls is 5-20 nm and allow small molecules to migrate. But bandwidth can vary, as additional “channels” are being formed in plants, through which water and nutrients dissolved in it are effectively transported inside the plant up to a certain concentration of nanoparticles.

Therefore, it is possible to speak about biocompatibility for NPs, which can be explained by such factors as: high adhesion to the surfaces of living systems, positive charge, size and shift of the pH of suspensions of nanoparticles:

Based on the analysis of the results of the studies, it can be concluded that positive NPs bind to negatively charged surface groups of mitochondrial membranes, neutralizing them. The transmembrane potential changes, the energy increases as well as the activity of synthesis of enzymes and phytohormones, IAA in particular. The protons of the mitochondria are pushed out, the proton pump is amplified, which is responsible for the removal of protons and their concentration increases in the outer layer of the membrane. The return through adenosine triphosphatase leads to the initiation of ATP synthesis, which stimulates the processes of growth and development. This factor suggests the indirect effect of nanoparticles on DNA synthesis.

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