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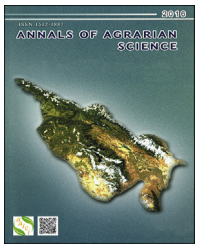

Peculiarities of ecotoxicological assessment nanoagrochemicals used in crop production

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ARTICLE INFO

Article history:
Received 10 January 2016
Accepted 29 January 2016
Available online 31 March 2016

Keywords:
Nanoparticles
Nanotoxicity
Nanotoksichnost
Bioassays on agrochemicals
Ecotoxicology

ABSTRACT

The article presents the results of studying the toxic effect of nanoagrochemicals on the processes of a plant's cell division, growth and development of plants at the early stages of ontogeny. It can be assume that the toxic effect of nanoagrochemicals depends on the size and structure of the nanoparticles, which are included in their composition: the toxic effect is stronger, the smaller the size of the nanoparticles is; nanocomposites of crystal structure are more toxic compared to nanocomposites of amorphous structure. Nanoagrochemicals ecotoxicological risk assessment should not be based only on the study of the dependence "dose-effect" on the level of the organism and population; it should include the research of the toxic process, starting from the level of the cell and its organelles.

Introduction

Today nanotechnology is increasingly used in various fields of human activity. One of the significant aspects is the application of nanotechnology solutions in agriculture, particularly, to optimize the conditions of supply of agricultural plants. Crop science is known for the use of nanopreparations, which can provide an increase in crop harvesting capacity by 1,5–2 times. Nanoagrochemicals differ from traditional agrochemicals as they contain nanoparticles (NPs). Nanoparticles are usually called particles sized from 1 to 100 nanometers (1 nm = 10^{-9} m) [1]. The increased interest of the researchers to nanoobjects is caused by the discovery of their unusual physical and chemical properties, especially, the biological actions that are often radically different from those of the same substance in the form of continuous phases or macroscopic dispersions. Known in the present biological effects of NPs can be divided into two groups: 1 – biocidal effect (meaning, the ability to kill living organisms), and 2 – the change of the functions of living organisms, which reveal itself in the biological objects of different levels of the organization. NPs, due to its small size, can bind to nucleic acids (causing, in particular, the formation of DNA adducts), proteins, get embedded in membranes, penetrate into cell organelles, and, thus, change the functions of biological structures. Due to the small size, nanoparticles cannot be recognized by the organism's defense system and are not subject to biotransformation and excreted.

According to experts of the Royal Society and the Royal Academy of Engineering of Great Britain, environmental hazards studies of nanomaterials are far behind the development of the nanotechnology industry itself [2,3]. This is completely related to nanoagrochemicals, used in agriculture. Typically, a dose of their application is lower than for conventional drugs (nanopreparations are used in mg/ha,

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Peer review under responsibility of Journal Annals of Agrarian Science.
http://dx.doi.org/10.1016/j.aasci.2016.03.002

traditional medicines – in kg/ha). This factor is often the main argument for a positive decision of ecological expertise as the basis for the calculation of risks is a classic dependence "dose-effect". However, in case of nanoagrochemicals it is necessary to consider not only the dosage but also such physical and chemical characteristics as size of the NP and its structure. They can condition the low NP's bioavailability, rate of achievement of a target structure, binding energy with bio-receptors and, consequently, the level of danger of the drug [4,5].

Objectives and methods

Studying the toxicity of nanoagrochemicals has been carried out at different levels of biological systems organization; objects were nanoagrochemicals, which include NPs:

Nano-Gro – a preparation, which is a water-soluble granule with diameter of about 4 mm, a weight of 0.05 g with a mass fraction of active components of sulfates of iron, cobalt, aluminum, manganese, nickel, silver $2.84 \cdot 10^{-9}$% granules or $1.43 \cdot 10^{-11}$ g.

Avatar-1 – a preparation, which is a colloidal solution of ultrapure carboxylates of natural food acids and especially pure biogenic metals of nanosizes (1–100 nm) in deionized water of 99.99999% purity.

The concentrations, which meet the applicable doses in the field conditions has been studied. The dose taken as the basic, which was recommended by the manufacturer for the dressing of agricultural crops, was being increased by 2–4 times:

Variant		Dose, mg/ha		Concentration of the solution, %
Control	1	–	–	–
Nano-Gro	2	100 (recommended)	1 RD	0.05
	3	200	2 RD	0.10
	4	300	3 RD	0.15
	5	400	4 RD	0.20
Avatar-1	2	50 (recommended)	1 RD	0.025
	3	100	2 RD	0.050
	4	150	3 RD	0.075
	5	200	4 RD	0.100

Defining the size, structure and chemical composition of the NPs of agrochemicals was performed by scanning electron microscopy (SEM Tescan Mira LSU 3 with a spatial resolution of 1 nm).

The study of mutagenicity, mitosis modifying and cytotoxic effects was carried out by applying ana telophase method on onion cells (*Allium cepa* L.). Mitosis modifying effect (changes in the mitotic activity of meristem) as a response of the test – system on the effect of nanopreparations determined by using the ratio of the mitotic index (MI).

The inhibitory effect of nanoagrochemicals towards the growth and development of certain plant organs were studied on the basis of cress (*Lepidium sativum* L.), according to ISO 11269-1:1993.

Results and analysis

It is known that nanoparticles have a so-called "quantum size effect". These effects are caused by the fact that with a decrease in size and the transition from the macroscopic body to the scale of a few hundred or a few thousand atoms, the density of states in the valence band and the conduction band varies sharply, which is reflected in the physical and chemical properties due to the behavior of electrons. There is an increase in the relative proportion of the "surface" atoms in different conditions than the bulk phase atoms (the coordination number, the symmetry of the local environment, etc.). The depth of the interaction of these particles with the environment is determined by two main factors: the surface energy and chemical nature of the NPs.

Results of the scanning electron microscopy showed that the size of the NPs of the preparation Avatar 1 were within 45.5–25.8 nm, preparation Nano-Gro – within 90 nm (Figs. 1 and 2). Chemically, the NPs of 1 the preparation Avatar-1 is a complex of chemical elements (%): C – 26.7, O – 17.5 Mg – 2.5, Al – 0.15, Si – 43.6, S – 0 04, Cl – 1.24, Mn – 0.24, Zn – 0.56, Ag – 7.27. The composition of the preparation Nano-Gro consisted of (%): C – 35.08, O – 14.51, Al – 0.10, Si – 42.21, S – 0.11, Ca – 0.10, Fe – 7.61, Cu – 0.29.

Many studies [1–5] reveal that the NPS, due to their small size, can penetrate into the cell organelles, and, thus, change the function of biological structures.

Results of studies of the cytotoxic effect on cells of *A. cepa* L. indicate that the use nanoagrochemicals in doses recommended for agricultural production, led to a decrease in the mitotic activity and to the change of the duration of the separate phases of the mitotic cycle (Table 1).

Mitosis modifying effect, which appears in the change of the mitotic activity of meristem and is a response to the action of nanoagrochemicals, was defined by the size of the mitotic index (MI). The MI was calculated (‰) as the ratio of the number of cells in the mitotic division of the total number of cells that are analyzed and calculated according to the formula (1):

$$MI = \frac{P + M + A + T}{P + M + A + T + I} \cdot 1000, \quad (1)$$

where P – a number of cells in prophase, M – in metaphase, A – in anaphase, T – in telophase, I – winterphase.

Preparations, by changing the relative duration of the phases of mitosis, influenced the development and formation of the unit cell division and thereby induced a mutagenic response. It was found that nanoagrochemicals, increasing the duration of prophase and metaphase, reduced the duration of meta- and anaphase.

Along with the typical cell division on the stage of ana- and telophase in the control variant (Fig. 3), mutations have been reported, which have appeared due to the nanoagrochemicals' effect and were associated with gross disruption of the structure of chromosomes, damage of the mitotic spindle (division spindle), and change in chromosome behavior on the division spindle (Figs. 4,5).

Under the influence of the preparation Avatar-1 the backlog of chromosomes division in anaphase and telophase