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INFLUENCE OF NANOPARTICLES (TI, NI, SI) ON INDICATORS OF INDUCED MUTATIONS OF *DROSOPHILA MELANOGASTER*

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ВПЛИВ НАНОЧАСТИНОК (TI, NI, SI) НА ПОКАЗНИКИ ІНДУКОВАНИХ МУТАЦІЙ *DROSOPHILA MELANOGASTER*

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ABSTRACT

In recent years, interest in the role of nanoparticles has increased significantly, in particular, their ability to influence mutagenesis is being studied. Studying the effect of nanoparticles on the functioning of eukaryotes using the example of a test object *Drosophila melanogaster* Meigen is used to assess the possible ecological consequences of their practical use.

The purpose: to investigate the effect of nanoparticles of Titanium, Nickel and Silicon on the features of development and mutagenesis in *D. melanogaster*. The object of the study: peculiarities of the development of flies of the species *D. melanogaster* line Canton S. The subject of the study: the effect of nanoparticles of Titanium, Nickel and Silicon on the development of flies of the species *D. melanogaster* line Canton S.

Methodology. To assess the genetic variability of experimental populations of drosophila, standard methods of accounting for the dynamics of adaptation were used based on numbers and sex ratios; fertility; statistical methods of information processing.

The scientific novelty of the work lies in the fact that for the first time the effect of Titanium, Nickel and Silicon nanoparticles on the occurrence of mutations in *D. melanogaster* and general biological indicators of animals.

Conclusions. The main requirements for nanoparticles in relation to their use in industry and provenance are: low or no toxicity, high biocompatibility, the ability to biodegrade or be removed from the body naturally. With the presence of nanoparticles in the nutrient medium, mutations were observed in flies: reduced wings and a discolored body and additional antennae and an elongated proboscis. In the second generation, the percentage of mutant individuals becomes smaller compared to the first generation. This is explained by the induction of repair systems in individuals that were developing in the presence of the investigated substances. In all recorded cases of mutagenesis, the number of females is statistically significantly greater than the number of males. When studying the mutational effect of nanoparticles in the offspring of both generations (F1 and F2), differences in the number of mutant individuals in males and females under the influence of the same substances are unlikely. Titanium nanoparticles have a more pronounced mutagenic effect. Taking into account the percentage value of mutations, we can conclude that there is a positive correlation between the concentration of the studied substances and the quantitative indicators of mutations.

Key words: *Drosophila*, induced mutations, mutational effect, nanoparticles

АНОТАЦІЯ

Останніми роками помітно посилився інтерес до ролі наночастинок, зокрема вивчається їх властивість впливати на мутагенез. Вивчення дії наночастинок на функціонування еукаріот на прикладі тест–об'єкту *Drosophila melanogaster* Meigen використовується для оцінки можливих екологічних наслідків за їх практичного використання.

Мета роботи: дослідити вплив наночастинок Титану, Ніколу та Силіцію на особливості розвитку та мутагенез у *D. melanogaster*. Об'єкт дослідження: особливості розвитку мух виду *D. melanogaster* лінії *Canton S*. Предмет дослідження: вплив наночастинок Титану, Ніколу та Силіцію на розвиток мух виду *D. melanogaster* лінії *Canton S*.

Методологія. Для оцінки генетичної мінливості експериментальних популяцій дрозофіли використовували стандартні методи обліку динаміки пристосованості за показниками чисельності та співвідношення за статтю; плодючості; статистичні методи опрацювання інформації.

Наукова новизна роботи полягає у тому, що вперше вивчено вплив наночастинок Титану, Ніколу та Силіцію на виникнення мутацій у *D. melanogaste*r та загальнобіологічні показники тварин.

Висновки. Основними вимогами до наночастинок стосовно використання їх у промисловості та пробуті є: низька або відсутня токсичність, висока біосумісність, здатність до біодеградації чи виведення з організму натуральним шляхом. За присутності наночастинок у поживному середовищі у мух спостерігались мутації: редуковані крила і знебарвлене тіло та додаткові антени і видовжений хоботок. У другому поколінні відсоток мутантних особин став меншим порівняно із першим поколінням. Це пояснюється індукцією систем репарації у особин, які проходили розвиток за присутності досліджуваних речовин. У всіх зафіксованих випадках мутагенезу кількість самок статистично достовірно більша за кількість самців. При вивченні мутаційного впливу наночастинок у нащадків обох поколінь (F1 та F2) відмінності чисельності мутантних особин у самців та самок за дії однакових речовин не вірогідні. Наночастинки Ті мають більш виражену мутагенну дію. Враховуючи процентне значення мутацій, можемо зробити висновок про позитивну кореляцію між концентрацією досліджуваних речовин та кількісними показниками мутацій.

Ключові слова: дрозофіла, індуковані мутації, мутаційний вплив, наночастинки

Formulation of the problem

In the near future, nanotechnologies are capable of making a revolution in science and society, which exceeds the consequences of the widespread use of computers. Many world and Ukrainian scientists associate the future leap in industry and other high-tech spheres of activity with nanotechnology (Chekman, 2009; Chekman, 2011).

Nanotechnology is actively used in the modern world, but little is known about its health risks. Nanoparticles are used not only in cosmetology and the production of detergents, but also in the food industry. Tiny particles are able to penetrate the body through the respiratory tract, gastrointestinal tract and, as is suspected, through the skin into the blood, pass the placental barrier, enter cells and even into the cell nucleus, that is, they can damage cells and genetic material (Syvolob et al., 2018).

At the moment, there are warnings about the risks of using nanotechnology in the cosmetics industry and in the production of clothes. As long as their impact on humans and the environment remains unstudied, products that can release these small particles should be avoided, the agency recommends. It is also necessary to introduce mandatory labeling for them, some politicians and scientists call (Trakhtenberh, 2013).

This determines the relevance of studying the effect of nanoparticles on the functioning of eukaryotes using the example of the test object *Drosophila melanogaster* Meigen to assess the possible ecological consequences of their practical use, however, preliminary study of the toxicity of the studied substances is expedient. Interest in the role of nanoparticles in the body has grown significantly in recent years. Some of the nanoparticles have already become an integral part of diagnostic procedures (Protsenko & Kozeretska, 2006).

For example, iron oxides with magnetic properties are used in magnetic resonance imaging (Chekman, 2011); gold nanoparticles, due to their photothermal characteristics, are used to destroy malignant cells. In general, a huge number of scientific works in the direction of nanotechnology are devoted to the diagnosis and treatment of malignant neoplasms (Trakhtenberh, 2013).

The purpose of the work: to investigate the effect of nanoparticles of Titanium, Nickel and Silicon on the features of development and mutagenesis in *D. melanogaster.*

Materials and methods

In the study, we used the pure lines of *D. melanogaster* which is maintained at the Department of Biology of the T.H. Shevchenko National University "Chernihiv Colehium". The experiment was conducted in June – December 2021, the sample size was about 1100 sexually mature individuals with dominant manifestations of eye color, wing shape and color of the body. The number of male and female individuals varied depending on the conditions of the experiment from 50 : 50 to 43 : 57. There were 10 individuals in each group (control and each of the experimental ones) at the beginning of the study. The number of individuals during the experiment depended on the conditions of creation and is indicated in Table 1.

The ingredients for the nutrient medium were weighed on technical and chemical scales. An appropriate volume of cold water was added to the agar weight and, with occasional stirring, brought to a boil and the agar dissolved. Crushed yeast was added to the boiling solution, brought to a boil and, stirring constantly, boiled over low heat for another 40 minutes. The loss of solution volume (evaporation of water) was compensated by adding hot water as it boiled. After that, sugar and semolina were added, brought to a boil and continued to boil for another 15 minutes. Propionic acid was added to the nutrient medium at the rate of 2 cm^3 per 1000 cm^3, cooled to 60-70 °C and poured into prepared vials with a layer of 1-1.5 cm using a laboratory funnel with a diameter of 15 cm. After complete cooling, yeast inoculation (lubrication) was carried out. Nanoparticles were added in the form of solutions on the surface of the nutrient medium in test tubes.

Flies of the basic and experimental cultures were developed and kept at a constant temperature (25 °C) and an adjustable photoperiod (12 : 12, day : night). The relative humidity was 60 – 70 %. *D. melanogaster* is characterized by sexual dimorphism, which greatly facilitates the identification of males and females when crossing and analyzing the offspring.

Each population group was a wild type of *D. melanogaster* and was characterized by a dominant manifestation of eye color, wing shape and body color. To determine the mutations that occur in individuals and to prevent modifications from being taken into account, the offspring of the 1st or 2nd generation were analyzed under the conditions of existence in the environment without the addition of the investigated substances.

To study the effect of nanoparticles on flies, nanoparticle substances were added to the nutrient medium in the form of solutions. The concentration of the solutions was 0.1 mg/cm^3 and 0.01 mg/cm^3, respectively.

Mutations were analyzed in 2 generations (Kimak–Holub et al., 2012). This was done to determine the phenotypic changes as mutational or modification. Statistical processing of the results was carried out according to general standards using the “Excel” program from the “Microsoft Office–2003” package and the STATISTICA 6.0 program.

Nanoparticles of Titanium, Nickel and Silicon were taken as the investigated substances, proposed by the senior researcher of the laboratory of virology of the Institute of Agricultural Microbiology and Agro-Industrial Production of the National Academy of Sciences, PhD of biology Derevyanko Stanislav and the graduate student of the Institute of Agricultural Microbiology and Agro-Industrial Production of the National Academy of Sciences Vasilchenko Anatoly.

Results and Discussion

When analyzing the number of adults and the sex ratio under the influence of the studied substances, it was established that the substances have different effects on the sex ratio (Fig. 1).

In the first generation, in the presence of all substances except Ni, the number of females exceeds that of males – maximally due to the action of Ti nanoparticles and minimally due to the action of Si. What can be explained by the different degree of lethal effect of substances on the development of male individuals (heterogametic sex). When comparing the number of males in F1 and F2, it is noted that their number varies greatly. In F1, the maximum total number of males is observed under the action of Ti nanoparticles, and in the F2 generation of drosophila, it is equal to the control. One of the main factors that ensure the variability of *D. melanogaster* is environmental contamination with mutagens, which are found everywhere, often found in the products of human production. For example, these are medicines, dyes, cosmetics, insecticides and herbicides (Solodovnyk et al., 2011; Selivon et al., 2012).

In genetic toxicology, it is customary to talk not only about mutagens, but also about genetically active factors that have a mutagenic effect. They affect crossover, in particular, gene recombination or the induction of reparative DNA synthesis, which is accompanied by damage to genetic material (Solodovnyk et al., 2011; Selivon et al., 2012).

Earlier, we studied the influence of xenobiotics on the biological, biochemical and genetic parameters of *D. melanogaster* (Solodovnyk et al., 2011; Selivon et al., 2012).

Among the mutational changes most often encountered in the studied flies during two generations, the following prevailed: an elongated proboscis, reduced wings and a discolored body. Table 1 shows the mutations observed under the action of Titanium, Nickel and Silicon nanoparticles and the quantitative indicators of *D. melanogaster*, in which corresponding mutations were found according to articles.

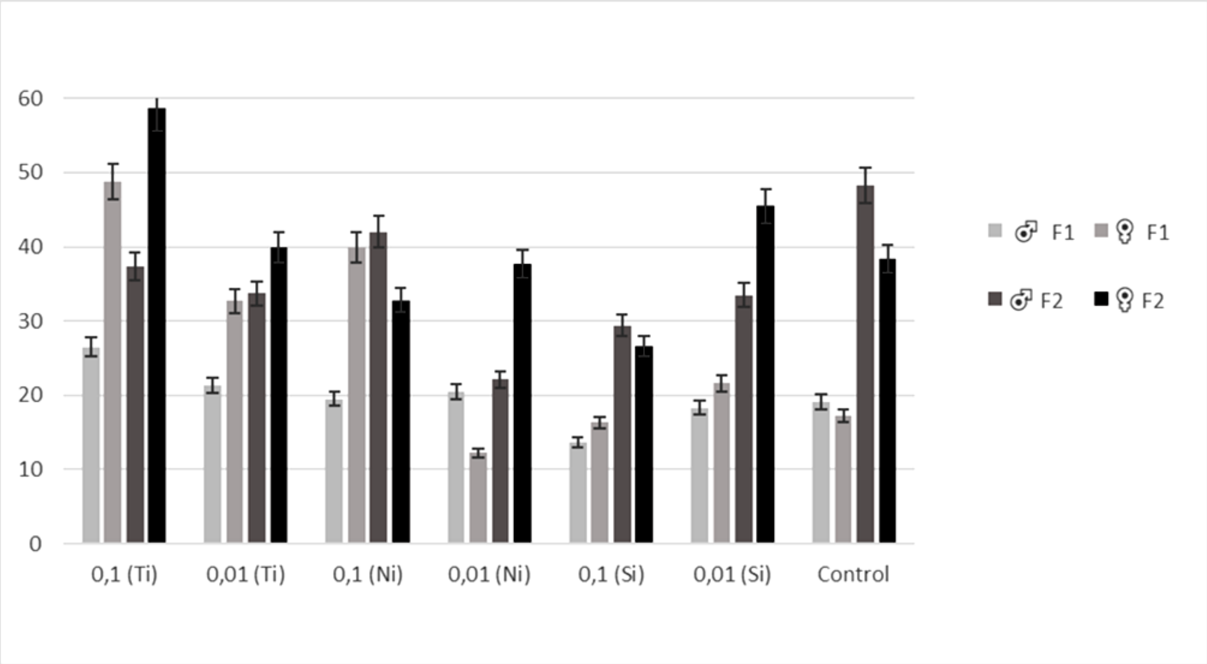

Fig. 1. The number of drosophila offspring under the action of nanoparticles of different concentrations

Table 1

Mutations of *D. melanogaster* discovered as a result of the experiment

Substance, concentration	The total number of individuals in the first generation	The number of mutations in the first generation	The number of individuals in the second generation	The number of mutations in the second generation
Ti 0.1 mg/cm³	202	31 ♀ and 15 ♂ (elongated proboscis)	240	34 ♀ and 17 ♂ (elongated proboscis)
Ti 0.01 mg/cm³	58	3 ♀ (reduced wings)	72	2 ♀ (reduced wings)
Ni 0.1 mg/cm³	83	17 ♀ and 11 ♂ (elongated proboscis)	134	9 ♀ and 5 ♂ (elongated proboscis)
Ni 0.01 mg/cm³	39	---	68	---
Si 0.1 mg/cm³	36	5 ♀ and 2 ♂ (completely white, without coloring)	65	8 ♀ and 3 ♂ (completely white, without coloring)
Si 0.01 mg/cm³	50	---	82	---
The control	75	---	115	---

We have analyzed the obtained data. The first thing we can see is that Ni and Si nanoparticles in a lower concentration did not cause mutational changes. This suggests that possible ways of using these substances in the pharmaceutical field can be investigated in more detail in the future.

At the same time, Ti nanoparticles in both studied concentrations and Ni and Si nanoparticles in a higher concentration caused mutational changes. Let's examine these mutations in more detail.

In all mutant groups, the number of mutant females is statistically significantly greater than the number of males ($P \leq 0{,}05$). Since there are more females, we can assume that such mutations are not related to sex. It is possible that certain forms of epigenetic imitation are present here, when heritable changes in gene expression are present. Such changes allow animals to adapt to changing conditions without changing the genome itself, but this issue requires more detailed research.

Ti and Ni nanoparticles in high concentration cause the same phenotypic mutations with approximately equal distribution by sex (elongated proboscis in 15 % of females and 7 % of males under the action of Titanium and 20 % of females and 11 % of males under the action of Nickel in the first generation). This may mean that the resulting mutations are identical or very similar.

Also, we note that all the obtained data turned out to be reliably significant. Taking into account the percentage value of mutations, we can say that nanoparticles in higher concentration have the greatest mutagenic effect. The fact that in the second generation we have almost no decrease in percentage, we can hypothesize that we have epigenetic inheritance (perhaps cytoplasmic).

In the second generation, the percentage of individuals carrying mutations decreases. This can be explained by the induction of repair systems in individuals that were developing in the presence of the substances under study. In all recorded cases of mutagenesis, the number of females is statistically significantly greater than the number of males. This allows us to make an assumption that these substances have a certain lethal effect on individuals of the heterogametic sex, which leads to a deviation of the sex ratio from the statistical one (1 : 1). But this question needs a more detailed study.

Conclusions

1. Analysis of literature data shows that nanoparticles have not only more pronounced pharmacological activity, but also toxicity compared to ordinary microparticles. The main requirements for nanoparticles in relation to their use in industry and provenance are: low or absent toxicity, high biocompatibility, the ability to biodegrade or be removed from the body naturally.

2. In the presence of nanoparticles in the nutrient medium, mutations were observed in flies: reduced wings and a discolored body and additional antennae and an elongated proboscis. In the second generation, the percentage of mutant individuals becomes smaller compared to the first generation. This is explained by the induction of repair systems in individuals that were developing in the presence of the investigated substances. In all recorded cases of mutagenesis, the number of females is statistically significantly greater than the number of males.

3. When studying the mutational effect of nanoparticles in the offspring of both generations (F1 and F2), differences in the number of mutant individuals in males and females due to the effects of the same substances are unlikely. Ti nanoparticles have a more pronounced mutagenic effect. Taking into account the percentage value of mutations, we can conclude that there is a positive correlation between the concentration of the studied substances and the quantitative indicators of mutations.

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