Stimulation of Seed Viability by Means of Dispersed Solution of Silver Nano-Particles

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***Abstract* —It was shown an essential influence of dispersed solutions of silver nanopowders on processes of seed germination and sprout growth of some grain and vegetable crops (winter triticale variety Ingen-93, winter wheat variety H335, spring wheat variety Arnautka 7, tomato varieties Iubileiny, Mikhaela, Surpriz). Under the impact of dispersed nanosilver solutions at concentrations of $32 \cdot 10^{-8} mg/l$, $16 \cdot 10^{-7} mg/l$, $8 \cdot 10^{-6}\, mg/l$, $4 \cdot 10^{-5} mg/l$ and $2 \cdot 10^{-4}\, mg/l$ at different exposure durations (30 min., 60 min. and 180 min.) it has been revealed a stimulation effect (by 10-30%) in some variants. The stimulation was observed in germination energy and viability of seeds and also in growth of aerial and underground parts of sprouts at different concentrations of the nanofactor. The task was to study the effect using dispersed nanosilver solutions at low concentrations. Processing the seeds infected by pathogenic fungi (specific to each genotype) with dispersed nanosilver solutions we have revealed that the nanofactor has more marked antifungal effect than solution of potassium permanganate commonly used for these purposes. The Data can be used in developing a method of pre-sowing stimulation of seed viability with dispersed solutions of metal nanopowders at low concentrations.**

***Index Terms* — antifungal effect, dispersed nanosilver solution, pathogenic fungi, potassium permanganate, stimulation of seed viability**

I. INTRODUCTION

Nanotechnology products such as metal nanopowders [1-6] began to be used more and more intensively in various fields of engineering, medicine and agriculture in the last decade. These nanomaterials arouse great scientific and practical interest in selectionists and plants geneticists as they have distinctly expressed antifungal effect on a vegetable object. Besides, they acts as microelements for mineral nutrition of plants of prolonged action increasing the adaptive potential of a vegetable organism (its efficiency and ecological stability) [2-6].

Metal nanopowders thanks to their extremely large specific surface area (about several hundred square meters per 1 g) can be effectively used in micro-doses. Thus, for a presowing processing of 1 t of seeds it is utilized only several milligram of nanopowder [3-5]. It provides the ecological safety both of the environment and of the bioproduct. Metal nanopowders toxicity is 10-40 times less than toxicity of salts of the same metals [3].

The impact of dispersed solutions containing metal nanopowders upon seeds of various plants leads to an essential increase of seed germination energy, their field viability, growth activity of sprouts and plants survival rate that positively affects all elements of crop structure. In this case wheat can increase its crop yield up to 15% and its green mass up to 25% and potatoes crop capacity rises up to 30%. Moreover sunflower, colza and mustard significantly raise their phospholipid concentration [1-6].

The biological effect depends on many factors: concentration of nanoparticles, nature of metal, duration of a single effect of nanoparticles on a vegetable object, form and size of nanoparticles, genotype of the object and its status, phase of plants ontogenesis, etc.

The optimal mode of stimulating effect of metal nanopowders on seeds and vegetating plants are little-investigated. None of the listed factors has been studied in greater detail and methodically competently. In this regard many questions concerning the mechanism of metal nanoparticles impact on a vegetable object still remain unanswered. In this work there was set the task to study how different concentrations of a dispersed solution of silver nanoparticles as well as different durations of a single effect of this solution influence on seed viability of some grain and vegetable crops.

II. MATERIALS AND METHODS

Objects of research*:* seeds of grain crops: winter triticale (Ingen-93 variety), winter wheat (H335 variety) and spring wheat (Arnautka 7 variety); seeds of vegetable crops: tomato (varieties Iubileiny, Mikhaela, Surpris). Plant varieties were created at the Institute of Genetics and Plant Physiology of the Academy of Sciences of Moldova and zoned in Moldova.

Factors of impact on the seeds: dispersed solutions of nanosilver at different concentrations: $32 \cdot 10^{-8} mg/l$, $16 \cdot 10^{-7} mg/l$, $8 \cdot 10^{-6}\, mg/l$, $4 \cdot 10^{-5} mg/l$, $2 \cdot 10^{-4}\, mg/l$; water suspension of *Helminthosporium avenae* (fungoid pathogen for seeds of cereals) and water suspension of *Alternaria alternata (*fungoid pathogen for seeds of vegetable crops). These fungi are main pathogens for seeds of cereals and vegetable cultures. They cause root rots at sprouts that significantly reduces crop yield and crop quality. Durations of pre-soaking the seeds in water suspension of fungi was 18 hours. As a disinfectant it was used 1% solution of potassium permanganate during 1 hour of pre-soaking the seeds. After pre-sowing processing the seeds were germinated in distilled water in Petri dishes at the temperature of 18-25° C in a thermostat. In each embodiment of the experiments 100-200 seeds were used. The following parameters were taken into consideration: the number of germinated seeds (that is, seed germinating ability), the sprout length, the main root length, the total dry mass of the sprout.

Nanoparticles of silver (NPAg) were obtained by reduction of silver ions in tea leaf extract at room temperature. The element analysis of the NPAg powder was implemented using an atomic absorption spectrometer Kvant-Z. The average silver grade in the nanopowder was 95-98%. Sizes of nanoparticles were defined with the help of a probe nuclear power microscope. NPAg nanoparticles were 35-60 nanometers in diameter.

III. RESULTS AND DISCUSSION

1. Assessment of the impact of the dispersed solutions of NPAg at different exposure concentrations and different exposure duration

The impact of NPAg nanoparticles on seeds of winter wheat (H335 variety) at different concentrations and at different exposure durations (expositions) didn't lead to any essential increase of seed viability and sprout lengths, but it was noticed some increase in the total dry mass of sprouts in some variants. Moreover, in a number of embodiments there was observed an essential decrease in the parameters. In our opinion, the low temperature during the sprouting process (+18^{o}C) could be a negative factor on the results.

In the next experiment with tomato seeds (Mikhaela variety) the same experimental conditions were applied but this time, the sprouting temperature was +25^{o}C. As it is seen from Table 1, the root length of has significantly increased as compared to the control at concentrations: $4 \cdot 10^{-5} mg/l$, $16 \cdot 10^{-7} mg/l$, $2 \cdot 10^{-5}\, mg/l$ and at expositions of 3 h., 1 h. and 30 min., respectively. These variants exceeded the control experiment by 7.6%, 10.0% and 9.8%, respectively.

Therefore, dispersed solutions of NPAg at different exposure concentrations and exposure duration had a positive impact on the bioeffect. In terms of technological effectiveness and profitability the best variant of NPAg concentration may be considered to be equal to: $16 \cdot 10^{-7}\, mg/l$ at 1 hour exposition.

2. Assessment of antifungal effect of NPAg nanoparticles on seeds of different varieties of grain and vegetable crops

Taking into account the above data, we used in the second part of the experiment one hour exposure duration and three exposure concentrations of NPAg: $8 \cdot 10^{-6} mg/l$, $16 \cdot 10^{-7} mg/l$ and $32 \cdot 10^{-8}\, mg/l$. The temperature of seed germination was 25^{o}C. In this part of experiment we first treated the seeds with the dispersed solution of nanosilver and then with the water suspensions of fungi culture.

In the experiment with triticale and wheat seeds (tab. 2) it has been revealed the influence of the genotype (variety) of the object on the bioeffect. The stimulation of growth by 11% was observed at the concentration of $16 \cdot 10^{-7} mg/l$ at a separate impact of the dispersed solutions of NPAg on triticale seeds. In the embodiment with winter wheat the growth stimulation was evident at two concentrations: $8 \cdot 10^{-6} mg/l$ (by 25%) and $32 \cdot 10^{-8}\, mg/l$ (by 25%). The stimulation was completely absent at seeds of spring wheat. The fungus negatively affected only the seeds of spring wheat (29.5%).

After a combined action of the dispersed solution of NPAg and of the water suspensions of fungi culture we have received some unexpected results. The growth of triticale sprouts after processing the seeds with fungal infection by nanosilver has even increased in comparison with control at all concentrations (by 4-15%). The intensification effect was also typical for the sprouts of winter wheat at two concentrations (by 18-26%). At the same time the dispersed solutions of nanosilver helped to overcome inhibiting action of the fungus in the seeds of spring wheat and the activity of sprout growth in the variant $8 \cdot 10^{-6} mg/l + fungus$ exceeded control by 12%.

Table 1. Length of the root of the eight-day sprout of tomato (Mikhaela variety) after the impact on its seed of the dispersed solution of NPAg nanoparticles in different concentration and at different exposure duration (exposition)

Exp., min	Concentration, mg/l	Root length, mm	Exp., min	Concentration, mg/l	Root length, mm
0	control	45.29 ± 0.85	0	control	45.29 ± 0.85
5	2x10^{-4}	45.58 ± 0.78	60	2x10^{-4}	37.89 ± 1.12***
	4x10^{-5}	45.74 ± 0.88		4x10^{-5}	46.79 ± 0.78
	8x10^{-6}	45.30 ± 0.68		8x10^{-6}	44.40 ± 0.75
	16x10^{-7}	43.54 ± 0.74		16x10^{-7}	49.86 ± 0.82***
30	2x10^{-4}	49.74 ± 0.79***	180	2x10^{-4}	46.96 ± 0.83
	4x10^{-5}	45.58 ± 0.84		4x10^{-5}	48.74 ± 0.82***
	8x10^{-6}	46.93 ± 1.69		8x10^{-6}	45.20 ± 0.73
	16x10^{-7}	44.06 ± 0.86		16x10^{-7}	41.32 ± 0.87**

Note: **, *** - distinctions are essential in comparison with control at significance values of 1% and 0.1%, respectively

Table 2. Length of the sprouts of cereals at separate and combined influence on their seeds of the dispersed solution of NPAg, the solution of potassium permanganate and the water suspensions of *Helminthosporium avenae*, mm

№	Variant	Triticale (Ingen-93)	Wheat (H335)	Wheat (Arnautka 7)
1	control	101.94 ± 3.49	75.47 ± 3.26	94.88 ± 2.90
2	fungus	96.56 ± 4.24	79.40 ± 3.06	73.30 ± 3.48
3	8x10^{-6} mg/l	91.53 ± 2.38	92.41 ± 3.10	96.83 ± 4.55
4	16x10^{-7} mg/l	113.13 ± 3.85	78.67 ± 2.79	98.22 ± 3.34
5	32x10^{-8} mg/l	81.43 ± 2.14	94.00 ± 5.90	90.09 ± 2.70
6	$KMnO_4$	97.67 ± 3.08	84.60 ± 3.48	88.60 ± 3.00
7	8x10^{-6} mg/l + fungus	107.60 ± 2.68	89.19 ± 3.78	111.54 ± 3.98
8	16x10^{-7} mg/l + fungus	117.02 ± 2.17	94.95 ± 2.85	101.89 ± 3.06
9	32x10^{-8} mg/l + fungus	105.60 ± 2.78	77.02 ± 2.81	69.78 ± 3.49
10	$KMnO_4$ + fungus	101,55 ± 2,81	106.50 ± 4.23	84.62 ± 2.24

Thus, sprouting of seeds of winter wheat at a higher temperature than in the first part of the experiment allowed to reveal a positive influence of the dispersed solution of nanosilver on the growth of sprouts and an evident stimulation was observed even at the lowest concentration of NPAg that is a prerequisite for continuation of the experiment with the use of still lower exposure concentrations of NPAg. It should be noted the existence of clearly expressed antifungal effect of NPAg on seeds of grain crops. At the same time, the traditional method of seeds' disinfecting by means of the solution of potassium permanganate turned out to be absolutely inefficient for the same seeds.

In the experiment with the seeds of different tomato varieties we got the following (tab. 3):

By the parameter *seed viability* it was revealed a pronounced influence of the genotype on the bioeffect. Viability of the seeds of Iubileiny variety has been considerably stimulated in all embodiments of the experiment, i.e. there was exhibited a favorable effect of the dispersed nanosilver solutions at three concentrations, of the water suspensions of fungi culture and of the solution of potassium permanganate as well as of the combined influence of the factors (excess over the control up to 90%). Viability of the seeds of Mikhaela variety has not changed significantly at the action of all the factors on the seeds. Viability of the seeds of Surpriz variety was stimulated in two variants: $32 \cdot 10^{-8} mg/l$ and $32 \cdot 10^{-8} mg/l + fungus$.

The root length of the sprouts of Iubileiny variety was stimulated at the impact of the dispersed nanosilver solutions on seeds at two concentrations: $8 \cdot 10^{-6} mg/l$ and $16 \cdot 10^{-7} mg/l$. The root length of the sprouts of Mikhaela variety was even a little inhibited. The root length of the sprouts of Surpriz variety has increased by 18% at the concentration of $16 \cdot 10^{-7} mg/l$ of the dispersed nanosilver solution.

The fungus negatively affected seed viability of all three genotypes. The solution of Potassium permanganate had a positive effect on the seeds of Iubileiny variety. The impact of the dispersed nanosilver solutions and the solution of potassium permanganate on the seeds before their infection by the fungus was inefficient. The length of the sprouts roots was very short in comparison with control and was at the level of the separate influence of the fungus.

Thus, it has not been revealed any correlation between the values of parameters *seed viability* and *root length* of the sprout. It becomes particularly obvious when comparing the impact on the seeds of fungus in separate and combined embodiments with NPAg. It is obvious that regardless of growth activity and resistance of the object to the pathogen *root length* parameter is more functional. The absence of inhibition in the embodiments with the fungus at initial germination of the seeds may be explained by the fact that a sprout uses the seed

Table 3. Seed viability and root length of sprouts of tomato varieties Iubileiny (I), Mikhaela (M) and Surpriz (S) after the seeds presoaking in the dispersed nanosilver solutions, in the solution of $KMnO_4$ and in water suspension of *Alternaria alternate*

№	Variant	Seed viability, %			Root length, mm		
		I	M	S	I	M	S
1	control	29	69	66	44.33	45.15	54.26
2	fungus	49	64	65	7.78	8.25	12.64
3	8x10^{-6} mg/l	44	56	69	48.94	44.23	50.29
4	16x10^{-7} mg/l	45	65	63	51.14	40.14	58.40
5	32x10^{-8} mg/l	51	62	78	46.84	39.31	54.61
6	$KMnO_4$	33	61	62	48.46	47.67	53.08
7	8x10^{-6} mg/l + fungus	40	67	65	9.61	7.90	8.60
8	16x10^{-7} mg/l + fungus	48	58	63	7.65	8.28	8.92
9	32x10^{-8} mg/l + fungus	56	51	77	10.69	7.69	10.31
10	$KMnO_4$ + fungus	55	60	62	15.47	13.31	12.05

nourishing substance during this period. The impact of the fungus manifests itself later when roots are being formed. Therefore the number of the sprouted seeds in this experiment cannot be regarded as an indicator of resistance to this particular fungus. The dispersed nanosilver solution in lowered concentration $32 \cdot 10^{-8} mg/l$ had a positive effect on the number of sprouted seeds. This parameter is very important when evaluating seed viability according to its germinating capacity. Therefore it would be rational, as with the seeds of cereals, to carry out experiments using still lower concentrations of the dispersed nanosilver solutions. The effect of fungus on the tomato seeds turned out to be so strong that we did not succeed in increasing of seed resistance with the help of the dispersed solutions of NPAg.

The experiences should be probably continued using shorter exposure duration of these funguses on seeds or using some other pathogens, some other concentrations of dispersed nanosilver solutions, other temperature experimental conditions, etc.

IV. CONCLUSION

1. It was observed an essential influence of the dispersed solutions of silver nanopowders on processes of seed germination and sprout growth of some grain and vegetable crops which is consistent with much of the data that has been reported earlier.

2. For the first time there were observed the following effects: stimulation of germination energy and of seed viability, growth of aerial and underground parts of sprouts after pre-soaking the seeds in the dispersed nanosilver solution with concentration of silver nanopowder up to $10^{-9} mg/l$. There are, apparently, still lower stimulation concentrations of nanofactor that should become a subject for further research.

3. It was noticed an evident antifungal effect of the nanofactor. As this takes place almost a complete disinfection of the seeds pre-processed with pathogenic fungi, specific for grain and vegetable crops (restoration of growth activity of experimental plants to initial control).

4. The obtained data can be used in developing of a perspective, cost-effective and ecologically pure method of pre-sowing stimulating treatments of seeds with dispersed solutions of metal nanopowders at low concentrations.

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REFERENCES

[1] I.P. Suzdalev, *Nanotechnology: Physical Chemistry of nanoclusters, nanostructures and nanomaterials*, Moskow, Komkniga, p.592, 2006

[2] *The main scopes of nanotechnology and nanomaterials in agrarian and industrial complex* //www.ferbanat.org

[3] G.E. Folmanis, N. V. Kovalenko, *Ultradisperse metals in agricultural production,* Moskow,1999

[4] A.P. Raykov, L.A. Panichkin, N.N. Raykova, *Nanopouders of metals – for ecologically safe agriculture* //www. Rusnanotech08

[5] N.N. Glushchenko, *Physical and chemical regularities of biological effect of high-disperse powders of metals,* Abstract of the thesis of the Doctor of biological sciences, Moskow,1988

[6] N.P.Egorov, O.D. Shafronov, D.N. Egorov, E.V.Suleymanov, *Development and carrying out of the experimental assessment of the efficiency of application in plant growing of new types of the fertilizers received with use of nanotechnologies*, Messenger of Lobachevsky State University of Nizhny Novgorod, No. 6, pp. 94-99, 2008