Bio-ecological consequences of crop seeds treatment with metal nano-powders

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Abstract. As a result of our investigations we have determined the optimal concentrations of ferrum, cobalt and cuprum nano-powders recommended to be used as micro-fertilizers increasing the yield and feed value of crops at the expense of accumulating biologically active combinations by 25-35 %. In unfavorable climate conditions, for example in a case of excess moisture or heat and drought, the plants development and ripening suffer. Our investigations have shown that the stimulating effect of nano-powders has lowered the effect of stress situations on plants development and simultaneously increased the rape seeds yield and quality. Treating the seeds with the drugs being studied has provided the high crop protection. If consider that the maximum efficiency of protectants Chinuk, SK (20 kg/t of seeds) and Cruiser, KS (10 kg/t of seeds) then for the same effect one needs nano-powders 0.1 g per hectare norm of seeds planting.

1. Introduction
The intensive agricultural production inevitably leads to the increased discharge of all soil mantle nutrients [1]. That is why compensation and support of the optimal balance of micro- and macro-elements in agrocoenosis are most important problems when getting the high yield of the quality [2]. It is known that many micro-elements seriously influence the agricultural products yield and quality and the decline of fungal and bacteria diseases injuriousness for plants [3].

High deficit and cost of traditionally used micro-fertilizers lead to the search for their new types therefore nowadays they study new generation substances – metals nano-powders that are viewed as stimulators of biochemical processes [4,5]. Nano-powders possess great possibilities in inorganic nutrition and energy deposition [6]. At the expense of uncompensated links they easily form complex compounds with organic ones resulting in different ferments synthesis and activation influencing carbohydrate and nitrogen metabolism, amino acids synthesis and reactions of photosynthesis and cells breathing. The results of our investigation prove that. The powders themselves with their energetic effect stimulate the processes of adaptation and self-organization of biological systems to the environment. Nano-particles decline environment negative influence to some extent.

The proposed metals nano-powders allow using them in agriculture as inexpensive and non-toxic drugs partially solving the problem of expensive bio-additives deficit and increasing the rate of return [7,8,9].

2. Methods
We have had phenological observations in field experiments, determined field germinating capacity and plants density. For this purpose we have detached permanent plots by the procedure of the State Crops Strain Test (1971) at every variant of the experiment. We have determined the plants height, herbage increment and accumulation of the dry matter collecting 100 plants from each variant. We have harvested at the plot discount areas, evaluated in metric quintal from 1 ha of the herbage.

We have chosen the plants to estimate the phytomass biological yield, to analyze the yield structure, to determine the dry matter and to conduct chemical analyses from the permanent plots (1 m$^2$) in the morning in dry weather after the dew drying (8-10 o’clock) and put them in poly bags with further temperature fixation for fermentative processes inhibition.

We have calculated the photosynthesis pure productivity or plants “net-assimilation” by formula [10]:

$$PhPP = \frac{M_2 - M_1}{1/2(L_1 + L_2) \cdot n},$$

where $PhPP$ – the photosynthesis pure productivity (g/m$^2$ · deliquefaction);

$M_1$ and $M_2$ – plants dry matter at the beginning and the end of the period;

$(M_2 - M_1)$ – dry matter increase during $n$ days;

$L_1$ and $L_2$ – leaves area at the beginning and the end of the period, m$^2$;

$1/2(L_1 + L_2)$ – average area of leaves during the experiment;

$n$ – days between sequential periods of observation time.

We have determined total nitrogen by Kjeldahl tritrimetry (GOST R 50466-93) and evaluated “raw protein” using coefficient 6.25; phosphorus by photometry (GOST 26657-97), kalium by flame-photometry (GOST 30504-97), calcium by chelatometry, cuprum, ferrum and cobalt by atomic adsorption (GOST 30178-96) using the extract 1n of HNO$_3$ solution for cobalt (CINAO, 1981), 1n of H$_2$SO$_4$ solution for ferrum, 1n for cuprum, fiber by Shtoman, i.e. the mixture of concentrated nitrogen and acetic acids (1:20).

We have determined mobile phosphorus and kalium in the soil by Kirsanov, pH of salt extract by potentiometer method; the sum of the exchange bases by Kappen-Gilkovits; the hydrolytic acidity by Kappen; humus by Tyurin; absorbed bases by trilonometry; mobile forms of cuprum and cobalt by the atomic adsorption method (muriatic and nitrate extracts correspondingly).

3. Results and discussion

The rape seeds pre-plant treatment with ferrum, cuprum and cobalt nano-powders in doses 0.030 g, 0.048 g, 0.120 g per hectare seed application norm has promoted the vivid increase of seeds germination energy by 6-9 % and the true increase of the lab germination has achieved 10 %. Thus, high-reactive nano-metals have participated actively in enzymatic processes of splitting some extra nutrients, providing their active germination. It should be noted that the higher level of the experiment plantlets metabolism processes has promoted the true increase of the lab germination as compared with the control. It allows considering the seeds pre-plant treatment with nano-powders as an important agronomic method of preparing the seeds for planting.

The seeds pre-plant treatment with ferrum and cuprum nano-powders has had approximately the same increase of field germination as compared with the control and has been from 5 to 8 %. We have found out the highest effect when treating the seeds with ferrum and cuprum nano-powders in the dose of 0.048 – 0.12 g/he.

The plants linear growth is an important parameter implicitly characterizing the intensity of cells division or expansion. We have discovered in our investigations that the seeds pre-plant treatment with ferrum, cobalt and cuprum nano-powders has promoted the increase of the rape plant height. In 2010 with extreme weather conditions (heat and drought) the plants had the highest height in a case with
nano-cuprum and that was 21-27 cm or 35.6-45.8 % higher than the height of the control ones (0.12 g/he). In the case with nano-ferrum only the dose of 0.48 g has given the height increase by 7 cm or 11.8 %. The pre-plant treatment with nano-cobalt has stimulated the rape growth promoting this parameter increase by 10-12 cm or 16.9-20.3 %.

In 2012 and 2013 being more favorable for growth and development all the experiment variants had activation of the plants linear growth. It has been most significant with nano-cobalt, not so much and approximately the same with nano-ferrum and nano-cuprum. On the average, for 3 years of investigations the highest excess of the linear growth as compared with the control has been discovered when using the nano-metals in the dose of 0.048 g. The rise to the control has been from 8 to 13 cm or 11-18 %.

Studying the influence of the seeds pre-plant treatment with nano-metals has shown that they can also influence positively the assimilating apparatus – leaf area fulfilling the leading role in photosynthesis. The difference in the control variant in these years has achieved about 7 thousand m$^2$/he. We have seen the highest excess of the leaf area as that of the control in the variant with nano-ferrum in all three doses and that has been from 5 to 7 thousand m$^2$/he.

We have got the maximum rise in a case with seeds treatment with nano-ferrum in the dose of 0.048 g and nano-cuprum in the dose of 0.03 g per hectare seed application rate (Table 1). It is known that even the short unfavorable influence of this or that environment already at the beginning of the seeds germination period causes considerable biological changes influencing later the yield. Therefore, one can suppose that seeds treatment with nano-metals can change the metabolism process and its intensity increasing its activity during all ontogeny. For 4 years of investigations the herbage maximum yield increase has been 65.0-56.7 c/he or 30.4-27.0 % as affected by nano-ferrum and nano-cuprum in the dose of 0.03-0.048 gr/hectare seeding rate (chosen as optimal). The nano-metals have not only changed the dynamics of the herbage increase but also promoted the plants chemical composition change. The seeds pre-plant treatment with all the metals in doses of 0.03 g and 0.048 g has promoted the protein increase by 40-47 % depending on the metal. The variants with nano-cobalt treatment have had the highest protein. It shows that cobalt participates in nitrogen and protein metabolism promoting the protein synthesis.

We have discovered that the variants have not had considerable differences in phosphorus and calcium. At that the content of microelements used for treating the seeds has not practically changed. So ferrum has varied in the range of 51.0–55.5 mg/kg of the dry matter in the experiment variants and 53.4 mg/kg in the control one. Cobalt in the experiment variants has varied in the range of 0.12–0.17 mg/kg of the dry matter and 0.11 mg/kg in the control one. The content of ferrum and cobalt in the soil before the harvest has been 17.3 mg/kg and 1.33 mg/kg correspondingly and 17.36 mg/kg и 1.35 mg/kg after harvesting that has not exceeded the control figures (17.30 mg/kg for ferrum and 1.23 mg/kg for cobalt) and ODC.

| Table 1. Influence of seeds pre-plant treatment on rape herbage yield (c/he) |
| Variants                  | 2009 | 2010 | 2011 | 2013 | Average Increase for 4 years |
|----------------------------|------|------|------|------|-----------------------------|
| Control                    | 181  | 152  | 257  | 249  | -                           |
| Nano Fe, 0.012 g           | 258  | 157  | 288  | 271  | 33.7                        | 16.0 |
| Nano Fe, 0.03 g            | 252  | 235  | 308  | 299  | 63.7                        | 30.4 |
| Nano Fe, 0.048 g           | 273  | 224  | 303  | 299  | 65.0                        | 30.9 |
| Nano Co, 0.012 g           | 237  | 225  | 270  | 268  | 40.2                        | 19.2 |
| Nano Co, 0.03 g            | 249  | 214  | 288  | 297  | 52.2                        | 24.9 |
| Nano Co, 0.048 g           | 247  | 214  | 285  | 295  | 50.5                        | 24.1 |
| Nano Cu, 0.012 g           | 236  | 200  | 283  | 266  | 36.4                        | 17.4 |
| Nano Cu, 0.03 g            | 247  | 231  | 296  | 292  | 56.7                        | 27.0 |
| Nano Cu, 0.048 g           | 245  | 215  | 290  | 289  | 49.9                        | 23.8 |
Investigation of the Prolonged Effect. The seeds harvested in 2009 were treated with nano-ferrum in the dose of 0.03 g per hectare seeding rate and later were planted in 2010 without nano-powders treatment. The yield was 203 c/he that exceeded the control by 14 % but was 27 % less than the yield got from the seeds treated with ferrum nano-powder in 2010. The yield increase is explained by higher quality seeds having more nutrients. The seeds harvested in 2010 were planted in 2011 without any nano-powders treatment. The yield has not differed from that of the control that shows reducibility of the ecosystem (Le Shatelye principle) to preserve homeostasis.

Later we have studied the higher concentrations nano-particles influence on the rape as we have planned to use the nano-metals to protect the plants decreasing herbicides to be used [11]. At such concentrations we have specially controlled microelements in the soil.

At the experiments with the rape seeds pre-plant treatment with metals nano-powders we have not discovered any authentic change of ferrum and cuprum as well as other chemical elements in the soil. These data prove ecological safety of the agricultural seeds pre-plant treatment with nano-powders (Table 2).

Table 2. Soil macro- and microelements when rape seeds treatment with metals nano-particles

| Parameters                        | Control | Experiment |
|-----------------------------------|---------|------------|
| Before planting                   |         |            |
| pH                                | 5.5±0.05| 5.6±0.05   |
| P (phosphorus), mg/100 g of soil | 12.2±0.04| 12.0±0.04  |
| K (kalium), mg/100 g of soil     | 14.1±0.03| 14.3±0.04  |
| Humus, %                         | 3.2±0.01| 3.1±0.08   |
| Fe (ferrum), %                   | 5.0±0.5 | 4.8±0.04   |
| Cu (cuprum), mg/kg of soil       | 17.0±0.04| 16.7±0.02  |
| Co (cobalt), mg/kg of soil       | 1.2±0.04 | 1.3±0.05   |
| Zn (zinc), mg/kg of soil         | 0.9±0.03| 0.92±0.004 |
| Pb (lead), mg/kg of soil         | 14.1±0.06| 14.0±0.5   |
| Cd (cadmium), mg/kg of soil      | 2.1±0.04 | 2.0±0.3    |
| After rape harvesting            |         |            |
| pH                                | 5.6±0.05| 5.7±0.02   |
| P (phosphorus), mg/100 g of soil | 12.2±0.04| 12.3±0.05  |
| K (kalium), mg/100 g of soil     | 14.0±0.03| 14.4±0.08  |
| Humus, %                         | 3.3±0.05| 3.1±0.03   |
| Fe (ferrum), %                   | 4.8±0.08| 4.7±0.05   |
| Cu (cuprum), mg/kg of soil       | 16.8±0.04| 16.9±0.06  |
| Co (cobalt), mg/kg of soil       | 1.3±0.05 | 1.4±0.04   |
| Zn (zinc), mg/kg of soil         | 0.91±0.005| 0.93±0.003 |
| Pb (lead), mg/kg of soil         | 13.9±0.08| 13.5±0.09  |
| Cd (cadmium), mg/kg of soil      | 2.0±0.5 | 2.1±0.06   |

Note: -P ≤ 0.05

At the experiments with the rape seeds pre-plant treatment with metals nano-powders we have discovered some increase of the plants vegetative and generative organs (Table 3 and grain yield).

Table 3. Mass correlation change between rape organs depending on seeds pre-plant treatment

| Parameter                        | Control | Nano Fe | Nano Cu |
|----------------------------------|---------|---------|---------|
| HCP_{0.95}                       | 18.9    | 22.7    | 26.7    | 16.0    |

4
Agro-ecological investigation of metal nano-particles influence on biological objects, plants in particular, is not possible without analysis of biologically active substances including vitamins: ascorbic acid and carotene. Nano-particles toxic affect on plants could have led to their synthesis in tissues decline that could have proved the nano-materials suppressing affect on agrobiocenosis.

Rape seeds treatment with optimal concentrations ferrum and cuprum nano-particles has promoted the increase of vitamin C in the herbage by 25.8 % and 26.9 % correspondingly in reference to the control. Carotene accumulation in the herbage has increased under the treatment up to 28-29 % as compared with the control figures.

The rape herbage chemical composition has not had any considerable changes except nitrogen-free extractable substances. Their content under metals nano-particles influence has increased by 10-14 % as compared with the control (Table 4).

The ability of ultra-fine metals to accumulate both in plants and the soil is very important while analyzing agro-ecological aspects of nano-technologies in energy crops growing. Table 4 presents the mineral composition of rape seeds.

| Elements                        | Control | Nano Fe (0.1 g/he. seeding rate) | Nano Cu (0.1 g/he. seeding rate) |
|---------------------------------|---------|---------------------------------|----------------------------------|
| Ferrum                          | 55.40±0.09 | 54.20±0.01                     | 56.70±0.02                       |
| Cuprum                          | 7.10±0.01  | 7.40±0.06                       | 7.0±0.05                         |
| Cobalt                          | 0.15±0.003 | 0.16±0.002                      | 0.12±0.004                       |
| Cadmium                         | 0.03±0.001 | 0.02±0.001                      | 0.04±0.002                       |
| Lead                            | 0.40±0.03  | 0.70±0.03                       | 0.90±0.06                        |
| Arsenic                         | 0.17±0.004 | 0.11±0.004                      | 0.13±0.006                       |
| Zinc                            | 51.8±0.02  | 47.40±0.05                      | 46.70±0.05                       |
| Nickel                          | 1.40±0.06  | 1.70±0.03                       | 2.0±0.6                          |

Note: -P ≤ 0.05

Microelements and heavy metals in the rape seeds got with the use of ferrum and cuprum have not differed greatly from the control ones. We have not also seen any ferrum and cuprum accumulation in seeds of the experiment variants that proves the absence of cumulative properties of the studied nano-materials.

Comparative evaluation of efficiency of spring rape different dressers and nano-powders has been conducted field tests data-driven got at the farm “Stenkino”. The rape seeds have been pre-plant treated with nano-powders having the concentration of 0.1 g per hectare seeding rate. In the control we have soaked the seeds in d water. We have used not only cuprum nano-powder but copper oxide one having high antibacterial and fungicide activity.
High efficiency of rape protection in variants with dressers Chinuk, SK and Cruiser, KS is well known [2,11]. Therefore the possibility of nano-powders to act has been compared with dresser Cruiser, KS.

Table 5. Degree of crops damage by most dangerous pests and rape yield with dressers

| Variant       | Number of Most Dangerous Pests, spec./100 plants | Total damages, items per 100 plants | Yield, c/he | Saved yield, % |
|---------------|-------------------------------------------------|-------------------------------------|-------------|---------------|
|               | Crucifero flea beetles                          | Cabbage moth                       | Rape weevil |               |
| Nano Cu, 0.1 g| 11.1                                             | 9.1                                 | 8.0         | 28            | 23.0          | 16.6          |
| Nano CuO, 0.1 g| 10.0                                            | 8.8                                 | 7.8         | 24            | 22.6          | 15.7          |
| Cruiser, KS, 10 kg/t of seeds | 10.5                                           | 8.9                                 | 7.8         | 26            | 19.5          | 14.7          |
| HCP₀.₅        |                                                 |                                     |             |               | 2.2           |               |

Treating the seeds with all the drugs being investigated has provided the crop total protection. Cruiser, KS has been a little bit more efficient against the rape weevil though on the whole there have been few differences. We should point out that biological efficiency in 15 days of using Cruiser, KS has declined a little in comparison with the effect of nano-powders but in spite of the high level of pests’ development they have saved the yield considerably.

In the process of observing the season dynamics of harmful fauna population in rape and other agrocenosis we have discovered that the pests’ population has changed considerably at phonological phases of crops development [12]. The crops colonization with pests has begun in the third decade of April. Cruciferous flea beetles have appeared first. At gradual temperature increase their population has been increasing for two weeks before the rosette formation. From the budding phase their population density has declined considerably at the expense of hibernate species dieback. We have noticed the largest number of pests and pollinators in the period of cabbage blooming.

Cruciferous flea beetles have turned to be most harmful. They have damaged the plants at the most expose phase, i.e. sprouts that could lead to their loss. The rape yield has depended on the used insecticide and nano-powders (Table 6).

Table 6. Spring rape yield depending on insecticide

| Yield, c/he | Kinmix 0.2 l/he | Nano Cu, 0.1 g | Nano CuO, 0.1 g |
|------------|-----------------|----------------|-----------------|
| Control    | 14.2            | 18.9           | 23.5            |

The decline of total damages has happened mainly at the expense of cruciferous flea beetles decline.

Kinmix has proved to have a fast solid action and high efficiency. But it has got a serious drawback – resistance of organisms to this class of combinations after several repeated applications. The high biological efficiency of the seeds pre-plant treatment with nano-powders has remained till the middle of the crop blooming so we have not needed the repeated treatment with insecticides.

Slow development of the spring rape aerial portion in the first period of growth has declined its competetiveness to weeds. In the case with the seeds pre-plant treatment with nano-powders the powerful development of the aerial portion lets rape dominate over weeds. The weeds species composition at the experiment area during the experiments has been widely presented mainly by annuals.

The perennial weeds infestation has been presented by rhizome plants – quitch (Elytrigia repens), field horsetail (Equisetum arvense); burr plants – Canadian thistle (Cirsium arvense), field milk or
yellow thistle (Sonchus arvensis), field bindweed (Convolvulus arvensis), dandelion (Taraxacum officinale) and great plantain (Plantago major). We have pointed out that if herbicides have had their better effect at temperature higher than 10° C, high relative air and soil humidity, then nano-powders have been especially efficient even in dry periods.

The nano-powders have not reduced the weeds but increased the yield and the first sprouts sustainability and the weeds affect has been minimal. The rape crop density and sustainability has been 233-240 species/m². There has also been some increase in the crop density, pods number per plant, mass of 1000 seeds and photosynthetic parameters.

The use of nano-materials has promoted formation of the powerful and active leaf apparatus making possible to increase the amount of accumulating PhAR that has been a good condition for crops yield increase.

Table 7. Influence of herbicides on spring rape yield

| Without herbicides (Control) | Klocet 1.5 l/he | Trophy 1.0 l/he | Nano Cu, 0.1 g | Nano CuO, 0.1 g |
|-------------------------------|----------------|---------------|-----------------|-----------------|
| 18.6                          | 21.7           | 22.5          | 23.3            | 22.1            |
| Saved average yield, %        | 15.3           | 13.4          | 16.0            | 17.1            |

Thus, in today ecological situation full of problems we propose native highly-efficient nontoxic nano-drugs of wide application spectrum possessing the ability to protect rape, get good yields and quality seeds.

4. Conclusions

Nano-crystal metals having unique properties can be used as new generation bio-drugs. Besides they are cost-effective and influence the increase of crops yield. High efficiency of nano-powders as growth stimulators has been shown in this article by the example of rape. We have shown that nano-powders having optimal concentrations up to 0.1 g/he seeding rate are ecologically safe as they do not accumulate in neither plants nor soil and have no mutagenity. The maximum increase caused by their use with spring rape as compared with the control has been up to 25%.

When growing rape it is very important to protect the plants. Developing integrated systems of rape protection means minimal negative environment impact of protective measures and maximum mobilization of natural biotic factors to suppress hazardous organisms’ development. We have noticed high efficiency of the spring rape seeds treatment with nano-materials against the pollen beetle and other pests and the yield average increase has been 4.4-6.9 c/he as compared with that of the control.

The use of herbicides and nano-powders treatment speeds up the crop ripening for 1-2 days, increases parameters of the yield structure, promotes powerful and active leaf apparatus functioning, increases accumulating PhAR and as a result serves a background for the crops yield increase and this rape yield increase when the pre-plant treatment with nano-powders can be up to 22% with other parameters considerable increase.

The adoption of resource-saving technologies with nano-materials use will help to escape the arable lands physical properties deterioration, soil degradation caused by the fertilizers active effect, pesticides and agricultural engineering. They must become additions to agricultural methods including science based rotations, choosing cultivars resistant to diseases, systems of tillage, weed control and timely harvesting. The peculiarity of nano-particles chemical cooperation with liquid fluid is one of determining factors in stimulating plants development that gives possibility to use nano-powders as growth stimulators and plants protectors.

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