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Evaluation of the biological effect of Zn nanocarboxylates and MoS_2 nanoparticles on corn sprouts *(Zea mays* L.*)*

Abstract. If it is necessary to reduce the anthropogenic impact on agroecosystems in conditions of constant increase in the cost of resources – improving the technology of growing corn through the use of nanotechnologies is one of the prerequisites for increasing the yield of crops and their profitability. In this regard, the purpose of the study was to investigate the biological activity of Zn nanocarboxylates and MoS_2 nanoparticles at different concentrations for the germination, growth, and development of corn seedlings. An experiment was conducted on corn sprouts to determine the effect of Zn and MoS_2 nanoparticles. The germination rate and germination energy of seeds under the action of nanoparticles were determined in accordance with the requirements of the state standard DSTU 4138-2002. The study results were analysed by generally accepted statistical methods. It was established that MoS_2 nanoparticles when diluted at 1:10,000 (at a concentration of 700 mcg/l) show biological activity and cause an increase in the length of leaf blades and the length of the roots of corn seedlings by 35.0% and 100.0%, respectively. At other concentrations (1:100; 1:1,000) of Zn and MoS_2 nanoparticles, no significant effect on the size of leaf blades and roots was observed. According to the results of the Mana-Whitney test, in all variants, with the exception of the action of MoS_2 nanoparticles in a 1:10,000 dilution, the significance level of changes was not sufficient. Only in the variant with MoS_2 at 1:10,000 dilution, an increase in root length was statistically significant. There was no statistically significant effect in all the studied variants on the germination energy and laboratory germination of maize seeds. The practical value of the study is to assess the effect of drugs (Zn nanocarboxylates and MoS_2 nanoparticles) for foliar top dressing, a wide range of which is offered to agricultural producers, selection of effective concentrations for the growth, development, and yield of corn per grain

Keywords: root length, leaf blade length, laboratory germination, germination energy, nanotechnology

INTRODUCTION

Corn is one of the main grain crops grown in Ukraine. In 2021, the sown area of corn was 5,522.4 thousand hectares, and the yield of grain was 42,109.85 thousand tonnes [1], so improving the technology of growing corn and increasing its yield is an important task of agricultural science.

In the last decade, the effect of nanoparticles (NP) of various substances and various nanomaterials (NM) on corn plants has been actively studied in the world and in Ukraine in particular [2-4]. Studies on the effect of NPs and other NMs on corn concern a wide range of issues: the effect of nanoparticles on germination energy and seed germination [5-7], morphometric [7-9], physiological, biochemical, and biophysical [3; 5; 9] plant parameters, mechanisms of NP uptake and transport by

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plants [3; 4], phytotoxicity of NPs [6] and, importantly from a practical standpoint, their impact on corn yield [7; 8; 10]. The global scientific community widely discusses the significant prospects of introducing nanotechnologies to increase the corn yield [7; 8; 11].

NPs and other NMs are known to affect the germination energy and germination rate of maize seeds. Thus, niobium-containing nanocomposites increase the germination energy and laboratory germination of seeds of the Kharkivskyi 340 MV corn hybrid by an average of 10% [1], and γ-Fe_2O_3 nanoparticles at a concentration of 20 mg/l, significantly increase the germination index by 27.2% [3]. At the same time, CuO [4], Si [5], TiO_2, SiO_2, CeO_2, Fe_3O_4, Al_2O_3 ZnO, and CuO [6] do not have a statistically significant effect on the germination parameters of maize seeds. Another study found that Fe nanoparticles increase corn seed germination by 7.89-12.25% at concentrations of 3-5 mg/l; while Co nanoparticles increase germination by 4.86% at concentrations of 3 mg/l, but reduce by 1.68-7.77% at concentrations of 4-5 mg/l; Cu nanoparticles do not significantly affect germination at concentrations of 3 mg/l, increase germination by 25.74% at concentrations of 4 mg/l, and reduce by 13.43% at concentrations of 5 mg/l [7].

NPs and NMs affect the morphometric parameters of maize plants at different stages of development. Thus, niobium-containing nanocomposites increase the length of seedlings of the Kharkivskyi 340 MV corn hybrid and their root system by 50-70% [2]. γ-Fe_2O_3 nanoparticles at a concentration of 20 mg/l significantly increase the length of corn roots by 11.5%, but at concentrations of 50 and 100 mg/l, it is significantly reduced by 13.5 and 12.5%, respectively [3]. CuO nanoparticles at concentrations of 10 and 100 mg/l significantly reduce the length of the root system of maize seedlings by 55-85% [4]. Under the conditions of vegetation experiment, Si nanoparticles increased the length of maize roots during the first 20 days of observation, but in the future, the growth parameters for the action of Si NPs did not differ from the control [5]. Another study showed that CU nanoparticles increase the length and dry weight of the root system of maize seedlings [7]. In the conditions of field experiment, it was found that during the treatment of ZnO nanoparticles, the height of corn plants was significantly higher than the control by 20.90% at the 60th day after sowing and by 16.11% at the time of harvesting [8].

Some researchers note the toxic effects of NPs on maize plants. It was found that CuO and ZnO nanoparticles at concentrations of 2,000 mg/l significantly inhibit the growth of the root system of maize seedlings, reducing its length by 95.73% and 50.45%, respectively. ZnO, Al_2O_3, and CuO nanoparticles have a dose-dependent effect on the length of the root system of maize seedlings, while Al_2O_3 NPs have the lowest toxicity [6]. It is worth noting that the same study found that CuO nanoparticles are not toxic to corn seedlings at a concentration of 10 mg/l [6], which means that the toxicity of NPs of many substances strongly depends on their concentration and with correctly selected concentrations, NPs can be quite safe for use in various sectors of the national economy, including agronomy.

NPs and NMs affect corn yield and structure in field experiments. Thus, Cu nanoparticles increase the weight of 1,000 seeds of the LVN10 corn hybrid by 4%. The yield of the VN8960 hybrid corn under Cu NP treatment was 8,589 t/ha, while the control crop was 7,030 t/ha [7]. Foliar application of ZnO nanoparticles significantly increases the average number of cobs per plant by 7.51%, the average length of the cob by 18.58%, the average girth of the cob by 12.61%, the yield of cobs by 16.85%, and the weight of stubble by 16.85% [8]. It is also established that the TiO_2 nanoparticles increase the average number of seeds on the cob and grain yield. With TiO_2 nanoparticles, an almost linear relationship was established between these indicators with a correlation coefficient $r = 0.97$ [11].

Many Ukrainian researchers note the biological activity of nanoparticles in stimulating plant growth and develop nanobiological fertilisers. [12]. A positive effect on the sowing qualities of leguminous seeds of pre-sowing treatment with the microelement preparation Avatar-1, immunomodulators (growth stimulator) Iodis concentrate and Iodis concentrate + Se and colloidal solutions of NP metals was established. Significantly, by 4-8%, the use of the immunostimulator Iodis concentrate + Se increases the laboratory germination of seeds. The use of Mo and Mn nanoparticles contributes to improving the sowing qualities of seeds. It was found that the laboratory germination rate of soy seeds increases by 5%, beans – by 7%, lentils – by 12%. Pre-sowing treatment of legume seeds with NP solutions of Ce, Ge, Se, and Cu leads to inhibition of germination [13].

The authors of the study previously proved the growth-regulating activity of the composition of Se and I nanoparticles on winter wheat seedlings [14]. The biological activity of Ni nanoparticles was studied in [15]. In addition, it has been experimentally proven that Zn NPs, the composition of Se and I NPs, and the microelement preparation Avatar-2 Protection not only increase potato productivity and marketability [16], but also can significantly reduce the degree of potato damage by infectious diseases and the frequency of their detection [17]. However, the biological activity of most known nanoparticles in relation to maize plants has not been investigated.

The purpose of the study is to assess the effect of Zn nanocarboxylates obtained by ablation and MoS_2 nanoparticles, obtained by biological synthesis at different concentrations for the length of leaf blades and roots, and the germination energy of corn seedlings.

MATERIALS AND METHODS

The study was conducted in 2021-2022 in the laboratory "Soil Fertility and Protection" of the Department of Soil Science and Soil Protection named after Prof. M.K. Shykula of the National University of Life and Environmental Sciences of Ukraine and in the laboratory "Virology" of the Institute of Agricultural Microbiology and Agroindustrial Production of NAAS. The study uses zinc nanocarboxylates (colloidal solutions of Zn nanoparticles stabilised by citrate anions, which are obtained by erosion-explosive dispersion [18]), obtained from LLC "Nanomaterials and nanotechnologies" from Doctor of technical sciences V.G. Kaplunenko, and MoS_2 nanoparticles obtained by biological synthesis, obtained from LLC "Scientific and technical centre Bioquant". The concentration of Zn nanoparticles was 4 g/l, MoS_2 nanoparticles – 7 g/l. Nanoparticle dilutions 1:100, 1:1,000, 1:10,000 in sterile distilled water were prepared before use.

The study of the biological activity of NPs was carried out using the hybrid corn Limagrain LG 30.271, FAO 260, weight of 1,000 seeds – 310 g. Before laying the experiment, the seeds were washed.

Corn seeds (72 units for each variant) were washed and soaked for 5 hours in warm water. Then, in petri dishes, 18 seeds were laid out on filter paper with the plant embryo to the top and sprouted at a temperature of 25-26°C. The repetition was fourfold. Seedlings with a root length of 1-2 cm were used for further experiments.

To determine the effect of NPs, the root tips (the growth point in the meristematic zone) of maize seedlings were placed for 1 hour in the corresponding NP dilutions. Control seedlings were placed in water. After that, the sprouts were again placed in petri dishes on wet filter paper and placed in a thermostat at a temperature of 25-26°C. After 24 hours, the length of leaf blades and roots was measured in control and experimental variants [19]. The obtained data were processed statistically [20]. The experiment scheme is shown in Table 1.

Table 1. Experiment scheme

Variant	Repeatability	Number of seeds in repetition
Control (H_2O)	Fourfold	18
Zn nanoparticles, dilution 1:100	Fourfold	18
Zn nanoparticles, dilution 1:1,000	Fourfold	18
Zn nanoparticles, dilution 1:10,000	Fourfold	18
MoS_2 nanoparticles, dilution 1:100	Fourfold	18
MoS_2 nanoparticles, dilution 1:1,000	Fourfold	18
MoS_2 nanoparticles, dilution 1:10,000	Fourfold	18

Seed germination and germination energy under the action of Zn and MoS_2 nanoparticles were determined in accordance with the requirements of the state standard DSTU 4138-2002 [21]. Statistical analysis was performed in StatSoft STATISTICA 12 software suite. Nonparametric statistical methods were used for the analysis. In the analysis of descriptive statistics, the median, upper and lower quartiles, and interquartile spread were calculated; the statistical significance of the results was determined by the Mann-Whitney U test [20].

RESULTS AND DISCUSSION

When comparing corn sprouts of the control group with experimental ones, it was found that under the action of Zn and MoS_2 nanoparticles in variants with Zn 1:100, Zn 1:1,000, 1:10,000, MoS_2 1:100, and MoS_2 1:1,000 the length of leaf blades was 40.0, 60.0, 40.0, 60.0, and 30.0% less than in the control, respectively (Table 2).

Table 2. Effect of Zn and MoS_2 nanoparticles on the length of the leaf blades of corn sprouts (cm)

Variant	Average length of leaf blades, cm	Δ, %	Lower quartile	Upper quartile	Interquartile range	Significance, according to the Mann-Whitney U test, P
H_2O	1.0	0	0.30	1.50	1.20	–
Zn 1:100	0.60	-40.00	0.40	1.40	1.00	>0.96
Zn 1:1,000	0.40	-60.00	0.30	1.30	1.00	>0.50
Zn 1:10,000	0.60	-40.00	0.30	1.00	0.70	>0.51
MoS_2 1:100	0.40	-60.00	0.30	0.80	0.50	>0.12
MoS_2 1:1,000	0.70	-30.00	0.40	1.50	1.10	>0.93
MoS_2 1:10,000	1.35	+35.00	0.65	2.00	1.35	<0.05

In a dilution of 1:10,000 MoS_2 NPs, the length of the leaf blades of maize seedlings was 35.00% longer than in the control (Table 2).

According to the Mann-Whitney test, in the Zn 1:100, Zn 1:1,000, Zn 1:10,000, MoS_2 1:100 and MoS_2 1:1,000 variants, the changes did not have a sufficient level of statistical significance. That is, at these concentrations of Zn and MoS_2 nanoparticles, it cannot be reliably stated that they are toxic to corn sprouts. In the MoS_2 1:10,000 variant, an increase in leaf blade length was statistically significant (Table 2).

It was found that in the Zn 1:1,000 and MoS_2 1:100 variants, the root length was 30.00 and 20.00% less than in the control, respectively. In Zn 1:100 and Zn 1:10,000, MoS_2 1:1,000 and MoS_2 1:10,000 variants, the root length was greater than in the control by 20.0 and 50.0; 40.0 and 100.0%; respectively (Table 3).

Table 3. Effect of Zn and MoS_2 nanoparticles by the length of the roots of corn sprouts (cm)

Variant	Average root length, cm	Δ, %	Lower quartile	Upper quartile	Interquartile range	Significance, according to the Mann-Whitney U test, P
H_2O	0.50	0	0.40	1.00	0.60	–
Zn 1:100	0.60	+20.0	0.60	1.00	0.40	>0.57
Zn 1:1,000	0.35	-30.0	0.20	1.00	0.80	>0.34
Zn 1:10,000	0.75	+50.0	0.35	1.00	0.65	>0.98
MoS_2 1:100	0.40	-20.0	0.20	0.80	0.60	>0.08
MoS_2 1:1,000	0.70	+40.0	0.30	1.00	0.70	>0.61
MoS_2 1:10,000	1.00	+100.0	0.50	2.00	1.50	<0.02

According to the Mann-Whitney test in all variants except MoS_2 1:10,000, the significance level of the changes was not sufficient. Only in the MoS_2 1:10,000 variant, an increase in root length was statistically significant (Table 3). In the Zn 1:100, Zn 1:10,00, and Zn 1:10,000 variants, a decrease in seed germination energy was observed by 19.44, 22.22, and 30.56%, respectively (Table 4). In variants of MoS_2 nanoparticles in dilutions of 1:100, 1:1,000, and 1:10,000, an increase in seed germination energy was observed by 8.33; 2.78, and 2.78%, respectively (Table 4).

Table 4. Energy of corn seed germination under the action of Zn and MoS_2 nanoparticles (%)

Variant	Average germination energy, %	Δ, %	Lower quartile	Upper quartile	Interquartile range	Significance, according to the Mann-Whitney U test, P
H_2O	50.00	0.00	38.89	66.67	27.78	–
Zn 1:100	30.56	-19.44	22.22	36.11	13.89	>0.05
Zn 1:1,000	27.78	-22.22	19.44	41.67	22.22	>0.11
Zn 1:10,000	19.44	-30.56	16.67	38.89	22.22	>0.11
MoS_2 1:100	58.33	+8.33	44.44	63.89	19.44	>0.88
MoS_2 1:1,000	52.78	+2.78	47.22	55.56	8.33	1.00
MoS_2 1:10,000	52.78	+2.78	47.22	58.33	11.11	>0.88

Thus, the Zn and MoS_2nanoparticles do not affect the germination energy of corn seeds.

In experimental versions for processing corn seeds by breeding Zn nanoparticles 1:100, 1:1,000, 1:10,000, there was a decrease in laboratory similarity by 13.89, 11.11, and 19.44%, respectively. For action of MoS_2 nanoparticles for the same dilutions, an increase in germination was obtained by 11.11; 8.33, and 13.89%, respectively (Table 5).

Table 5. Laboratory germination of corn seeds by the action of Zn and MoS_2 nanoparticles (%)

Variant	Average germination rate, %	Δ, %	Lower quartile	Upper quartile	Interquartile range	Significance, according to the Mann-Whitney U test, P
H_2O	58.33	0.00	47.22	75.00	27.78	–
Zn 1:100	44.44	-13.89	38.89	50.00	11.11	0.20
Zn 1:1,000	47.22	-11.11	33.33	55.56	22.22	>0.34
Zn 1:10,000	38.89	-19.44	30.56	61.11	30.56	0.20
MoS_2 1:100	69.44	+11.11	61.11	77.78	16.67	>0.48
MoS_2 1:1,000	66.67	+8.33	61.11	77.78	16.67	>0.48
MoS_2 1:10,000	72.22	+13.89	63.89	80.56	16.67	>0.48

According to the Mann-Whitney U test in all the studied variants for the use of Zn and MoS_2 nanoparticles, the changes did not have a sufficient level of statistical significance. That is, at these concentrations of Zn and MoS_2 nanoparticles it cannot be said that they affect the laboratory germination of corn seeds (Table 5).

As a result of the conducted studies, it was established that the MoS_2 NPs in a dilution of 1:10,000 (at a concentration of 700 mcg/l) show biological activity and cause a statistically significant increase in the length of the leaf blades of corn seedlings by 35.0% and the length of the roots by 100.0%. Using Zn and MoS_2 nanoparticles at other concentrations, an increase in the length of leaf blades and roots was not detected, which is consistent with the results obtained by other scientists [7]. There was no statistically significant effect in all the studied variants on the germination energy and laboratory germination of maize seeds.

According to the results of studies by other researchers, nanoparticles of various elements, or nanoparticle compositions at different concentrations can be toxic to seedlings of seeds of different crops [7], reducing the size of leaf blades and roots, stimulating the growth and development of seedlings, increasing, reducing the energy of germination and seed germination, or not having a significant effect on these indicators. CuO nanoparticles at a concentration of 100 mg/l did not affect germination, but suppressed the growth of maize seedlings [4]. It was found that the phytotoxicity of ZnO, Al_2O_3, and CuO nanoparticles in the range from 25 to 2,000 mg/l depended on the nanoparticle concentration [6]. Thus, both the results of previous studies and the materials presented in the paper strongly indicate a dose-effect relationship for the studied NPs.

The dependence of phytotoxicity on the method of obtaining nanoparticles is established. It is known that under the action of Se nanoparticles obtained by ablation, the length of winter wheat leaf blades decreased by 15.84%, while under the action of Se Bio nanoparticles obtained by biosynthesis in the same concentration, their length increased by 40.32% [14], which indirectly indicates the prospects of using nanoparticles obtained by biosynthesis.

For the action of Ni nanoparticles at a concentration of 8 mcg/l, the length of leaves and roots was greater than in the control variant by 41.79% and 36.76%, respectively, however, there was no statistically significant effect of Ni nanoparticles on the energy and dynamics of germination of winter wheat seeds [15], which is consistent with the results of studies on corn seedlings.

Thus, the results obtained are consistent with the results of previous studies and indicate the prospects for using MoS_2 nanoparticles to improve the technology of growing corn.

CONCLUSIONS

It is established that MoS_2 nanoparticles at a lower concentration than 700 mcg/l and all studied concentrations of Zn nanoparticles reduced the length of maize leaf blades by 30.0-60.0% compared to the control variant. The increase in root length is statistically significant only in the MoS_2 1:10,000 variant. Seed germination during corn processing increased with the use of MoS_2 nanoparticles in subsequent dilutions 1:100, 1:1,000, 1:10,000 by 8.33-13.89%.

Zn and MoS_2 nanoparticles at all the studied concentrations do not significantly affect the germination energy and laboratory germination of seeds. According to the results of statistical processing using the Mann-Whitney test in all the studied variants for the use of Zn and MoS_2 nanoparticles, the changes did not have a sufficient level of statistical significance.

The obtained data from laboratory studies indicate high prospects for the use of MoS_2 nanoparticles in improving the technology of growing corn and the need for further field research on the development of nanofertilisers.

Further research will aim to evaluate other effective concentrations (1: 50,000; 1:100,000) of nanoparticles of MoS_2 and especially Zn (1:20; 1:50), since the effectiveness of using the latter on corn crops has not been proven.

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Оцінка біологічної дії нанокарбоксилатів Zn та наночастинок MoS_2 на проростки кукурудзи *(Zea mays* L.*)*

Анотація. За необхідності зменшення антропогенного впливу на агроекосистеми в умовах постійного здороження ресурсів – удосконалення технології вирощування кукурудзи за рахунок застосування нанотехнологій є однією з передумов підвищення урожаю культури та її рентабельності. У зв'язку з цим, метою дослідження було вивчити біологічну активність нанокарбоксилатів Zn та наночастинок MoS_2 за різних концентрацій на проростання, ріст та розвиток проростків кукурудзи. Для визначення впливу наночастинок Zn та MoS_2 проводили дослід на проростках кукурудзи. Схожість та енергію проростання насіння за дії наночастинок визначали відповідно до вимог державного стандарту ДСТУ 4138-2002. Результати дослідження проаналізовані загальноприйнятими статистичними методами. Встановлено, що наночастинки MoS_2 за розведення 1:10000 (у концентрації 700 мкг/л) проявляє біологічну активність та зумовлює збільшення довжини листових пластинок та довжину коренів проростків кукурудзи на 35,0 % та 100,0 % відповідно. За інших концентрацій (1:100; 1:1000) наночастинок Zn та MoS_2 суттєвого впливу на розмір листових пластинок та коренів не спостерігалось. За результатами тесту Мана-Уітні усі варіанти, за винятком дії наночастинок MoS_2 у розведенні 1:10000 рівень значущості змін був не достатнім. Лише у варіанті MoS_2 1:10000 зростання довжини коренів було статистично значущим. Не встановлено статистично значущого впливу в усіх досліджуваних варіантах на енергію проростання та лабораторну схожість насіння кукурудзи. Практична цінність наукової роботи полягає у оцінці впливу препаратів (нанокарбоксилатів Zn та наночастинок MoS_2) для позакореневого підживлення, широкий асортимент яких пропонується агровиробникам, підборі ефективних концентрацій на ріст, розвиток і урожайність кукурудзи на зерно

Ключові слова: довжина кореня, довжина листової пластинки, лабораторна схожість, енергія проростання, нанотехнології