Hoang *et al. Chem. Biol. Technol. Agric.* (2022) 9:12
https://doi.org/10.1186/s40538-021-00277-w

Chemical and Biological Technologies in Agriculture

LETTER TO THE EDITOR

Open Access

Evaluation of metal nano-particles as growth promoters and fungi inhibitors for cereal crops

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Abstract

Background: Nano-particles of metals can be routinely synthesized. The cereal seeds treatment with the particles can improve early growth and crop production. Moreover, the treatment is robust and economical.

Methods: Metal (Fe^0, Cu^0, Co^0), zinc oxide (ZnO) and chitosan-stabilized silver nano-particles were synthesized and applied to cereal seeds. The germination rate, early plant development and inhibition effects on pathogenic fungi were quantified.

Results: It was found that all nano-particles had a positive effect on the development of healthy cereal seedlings. In particular, the length of the above-ground part of the seedlings was increased by 8–22%. The highest inhibition effect was observed on *Helminthosporium teres* with the application of Co^0 and chitosan-Ag. Pre-sowing treatment with metal nano-particles reduced the number of infected grains by two times for wheat and 3.6 times for barley. The application also increases the chlorophylls and carotenoids in both uninfected and infected seedlings.

Conclusions: The results demonstrated a robust application of nano-particles in improving cereal production.

Keywords: Metal nano-particles, Pre-sowing treatment on germination, The toxicity of metal nano-particles to phytopathogenic fungi

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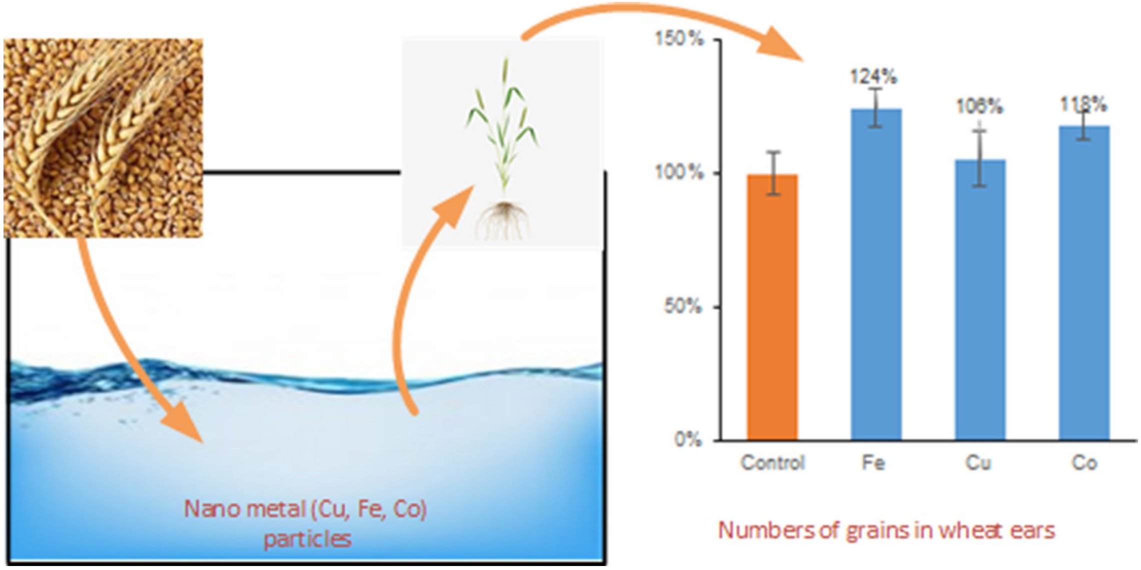
Graphical Abstract



Introduction

Food crops, such as wheat, rice, maize, barley, etc., are vital for the human population. With increasing population and limited arable land, increasing crop yield remains a priority for many countries. Nanotechnology is one of the latest technological advances of the twenty-first century, which is considered a critical improvement of crop productivity [1]. While nanotechnology has been successfully employed in materials engineering, the potential application in agriculture has not been realized [2, 3]. The small size of the nano-particles allows them to pass through biological membranes, accumulate in the internal environment, integrate into proteins and, subsequently, change biological functions. The range of tested nanomaterials in the literature is extensive [4, 5]. Many studies have corroborated the effect of metal nano-particles on plant growth and disease resistance [6]. However, the mechanism of the biological action of nano-metals remained poorly understood. The particles can be applied at different stages of crop development [7]. However, applying particles to the soaking solution prior to germination requires a small metal content and does not affect soil conditions [8].

The enhanced crop growth was evidenced with silver [9, 10], copper [11, 12], silicon [13] and iron [14] nano-particles. In addition to zero-valent metal particles, metal oxide particles, such as zinc oxide [15] and copper oxide [16], were also applied. The metal particles, such as iron, cobalt and copper, can release electrons upon dissolving into the water due to the high reduction potential. It is interesting to notice the selective impact of these metals. For instance, Cu^0 and Co^0 have promoted the plant body better than Fe^0 during the germination stage of maize seeds in the tropical climate [12].

Grains are a source of preservation of many pathogens, as they are rich in proteins, minerals and nutrients. Pathogens that persist in the seed lead to a decrease in seed germination, damage to the root system, and seedlings' death. In addition, sowing with infected grains can spread diseases to vegetating plants and thus create an infection in the field. Therefore, seed treatment is a necessary procedure for growing grain crops. A previous study has found that copper oxide particles can suppress infection in soybean [17]. Among the reported fungi, the saprotrophic mould fungi *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*, *Cladosporium* are dominant for cereal crops [18]. All of them are ordinary representatives of the surface microflora of grains. In addition, fungi of the genera *Bipolaris*, *Alternaria*, *Fusarium* were commonly found [19]. For barley, *Helminthosporium teres* is the most well-known pathogenic fungi and has been investigated over a century [20].

It is expected that the impact of metal nano-particles can vary with plants and climate. This study investigates the effect of metal nano-particles in pre-sowing treatment on the early growth of cereal crops in a temperate climate. Three metal nano-particles (Fe^0, Cu^0, Co^0) were synthesized [12] and used in the study. In addition to these zero-valent metals, stable metal oxide (ZnO) and organic-stabilized Ag particles were synthesized and employed. The germination and morphometric parameters at the early stages of wheat and barley grains were experimentally monitored. The extent of inhibition the particles on phytopathogenic fungi growth was also quantified. The study aims to clarify the influence and optimal conditions of metal nano-particles on cereal crops.

Methods and materials

Preparation and characterization of nano-particles

$Fe(NO_3)_3$, $Co(NO_3)_2$, $Cu(NO_3)_2$ were used as precursors for precipitating processes with NaOH solution. Three metals, iron, copper and cobalt, were synthesized via the reduction method at an elevated temperature. The optimal temperature for the particles was selected from the previous experiments: 400 °C for Fe and Cu, and 300 °C for Co. The reaction time was 90 min with a hydrogen flow rate of 350 ml/min. The final particle size was between 30 and 70 nm. The synthesized metal particles were immediately stored in double-side vacuum bags. A previous study has shown that these particles remain in a zero-valent state for up to 12 months. The phase structure of as-prepared nanoparticles was verified by X-ray diffraction and SEM as described before [12]. The percentages of the oxidized and metallic elements were calculated from XRF. The purity of the metallic content was greater than 99%.

For silver particle synthesis, the aqueous solution method was used [21]. The reaction of nanosilver formation was taking place in a homogeneous solution of $AgNO_3$ with β-chitosan as a stabilizer and permanent stirring at ambient temperature while the reducing agent ($NaBH_4$) was being added successively drop by drop. Chemical reagents were from Merck and Sigma Aldrich. β-chitosan was provided by the Institute of Chemicals (Vietnam). Stock solutions were prepared using bi-distilled water. For chitosan-Ag particles, FE-SEM was used to confirm the particles (Additional file 1: Fig. S4 and S5).

Plant material and pre-sowing treatment

The selected objects were seeds of spring wheat *Triticum aestivum L.* and spring barley *Hordeum vulgare L.* Spring wheat variety "Sudarynya" is a medium-ripe variety of Belarusian selection. Spring barley variety "Magutny", an early ripe variety of Belarusian barley, was selected. Both grains were used with permission from the commercial supplier ("Евро-Семена", Belarus). The study complies with the relevant national and international guidelines and legislation. Non-valence metal nano-particles (Fe^o, Cu^o, Co^o) and nano-zinc oxide (ZnO) are dispersed into double-distilled water and ultrasonically vibrated to create a suspension solution. The optimal ultrasonic time of 20 min was used [12]. Since chitosan-Ag was stable in an aqueous solution, it was used directly without sonication. The concentration of nano-particles used in the experiments was 0.3–0.5 mg/kg grain for Fe, Cu, Co, ZnO nano-particles and 15–60 mg/kg grain for Ag nano-particles.

Three experimental studies evaluated the effectiveness of particles. First, the germination and pathogen control were tested in the laboratory. Second, the fungicidal activities were tested by mixing the particles with a nutrient medium. Third, the impact of the particles on plant growth and production was evaluated by growing crops in the plant nursery. Statistical analysis was performed using Microsoft Office Excel by calculating the arithmetic mean and standard deviation. The Shapiro–Wilk test [22] was used to assess the normality of distribution. The reliability of the sample differences with a normal distribution was tested with the parametric Student's t-test. Significance was tested at a probability level of 95% or 99%.

Germination and pathogen control

Sowing characteristics of seed were determined according to the current Standards of the Republic of Belarus. The seeds were soaked in with freshly prepared solutions of particles for 8–10 h. The control samples were soaked in double-distilled water. Subsequently, they were dried and germinated in Petri dishes on filter paper moistened with distilled water in a thermostat at 21 °C. For each sample, 100 seeds were selected and replicated four times. Germination energy and germination rate were determined on the 3rd and the 7th day, respectively. On the 7th day, the seedling length, the main root length, and their mass were also quantified.

Pathogen infestation of cereal seeds was determined in laboratory conditions by germinating seeds on nutrient agar. The chemical composition of agar was the same as the growing culture (described below). A mycelia block (3 mm in diameter) of the pathogen at the actively growing edge of the culture was placed in the centre of a 5-cm-diameter Petri dish. Each treatment was repeated three times, with an equal number of seeds, on three different dishes. Diseases caused by fungi on germinated and non-germinated grains were manifested in the form of spots of various shapes and colours, mycelium plaque, deformation or dead parts of seedlings. Seed contamination with phytopathogenic fungi was expressed as a percentage of infected seeds to the total amount in the sample. In this experiments, the nanomaterial concentrations in the agar were the same as the concentration in the soaking solution, as mentioned above.

A more detailed investigation of seedlings infection was conducted with barley and the phytopathogenic fungus *Helminthosporium teres.* The seeds were infected with spores of *Helminthosporium teres* at the age of 10 days. On the sixth day after infection, when the infection is visible, the content of photosynthetic pigments, lipid peroxidation products and water-soluble content from healthy and infected plant tissues were determined [23].

Fungicidal activities

Fungicidal inhibition of the five nano-particles was tested with three phytopathogenic fungi—*Alternaria sp.*, *Fusarium sp.* and *Helminthosporium teres*. These three fungi groups were selected based on their relevance in cereal production [24]. The impact of metal nano-particles on the mycelium development of phytopathogenic fungi was tested on sterile nutrient aqueous solutions of nano-particles. The particle solution was prepared with double-distilled water at three different concentrations (Additional file 1: Table S1). Subsequently, lactose (at a concentration of 20 g/L) and urea (at a concentration of 1.2 g/L) were added as a source of carbohydrates and nitrogen, respectively. The vital minerals were also added: $MgSO_4$ (0.5 g/L), KCl (0.5 g/L) and KH_2PO_4 (0.5 g/L). Agar was used as a solid thickener. The mixed solutions (in a volume of 15 ml) were poured into Petri dishes. The nutrients and minerals were mixed with double-distilled water at the same concentrations to prepare the control medium.

Sterilization was carried out with steam at 1 atmosphere for 1 h [25]. After solidifying the medium, agar blocks (2 mm in diameter) with the mycelium of the studied fungi were placed in the centre of the dishes. Fungal cultivation was carried out at 25 ± 1 °C. The diameter of the grown colonies was measured on the 10th day. The inhibition of colony growth was quantified as the ratio of the diameter of fungi mycelium between the nanoparticles-added media and control medium.

Crop productivity

For crop productivity analysis, plants were grown on standard growing plots for each variant in triplicate, at the Institute of Experimental Botany (National Academy of Science, Belarus). The growing season lasted 80 and 90 days for barley and wheat, respectively. The effect of metal nanoparticles on the formation of grain crop productivity was investigated by quantifying the critical indicators of the plant, ears and grains. The crop structure was determined in the phase of wax ripeness in ten plants of each group in four replicates. The main elements of the crop structure were: (i) the height of the plant; (ii) the quantity of productive and unproductive stems; (iii) the mass of straw and ears from 10 plants; (iv) the weight and the number of grains in the ears; (v) the mass of 1000 grains.

Results and discussion

The influence of nano-metals on the growth of the cereal seedlings

The application of metal nano-particles in pre-sowing treatment led to a positive change in morphometric parameters for both wheat and barley (Fig. 1). Wheat seedlings were more sensitive to treatments. Except for chitosan-Ag, the concentration of the four nano-particles was between 0.3 and 0.5 mg/kg of grains. The aqueous concentration of chitosan-Ag was between 15 and 60 mg/kg, as detailed in Additional file 1: Table S1. The application of Cu^0, Fe^0 and Co^0 increased the length of the above-ground part of the seedlings in all concentrations. The range increment is between 14 and 22%. The most significant improvement of wheat seedling length was observed with Fe^0 at 0.4 mg/kg. This improvement was 22% and was statistically significant at a 1% level. ZnO and chitosan-Ag particles had a weaker effect, only from 6 to 10%. The particles did not produce significant improvement for barley (Fig. 1a and b). The height of the above-ground part of barley seedlings increased by 6–9% compared to the control sample (healthy seeds but untreated with any metals).

Notably, the decrease in germination energy and germination of Co^0-treated grains did not cause further inhibition of plant growth. Under the influence of Co^0 (concentration between 0.3 and 0.5 mg/kg), wheat and barley showed an increase in the seedling length of 12–14% and 6–9%, respectively.

Antifungal properties of metal nano-particles

The study of fungitoxicity of metal nano-particles began with their direct impact on the growth of mycelium phytopathogenic fungi (*Fusarium sp.*, *Helminthosporium teres*, *Alternaria sp.*), which cause root rotting, leaf spotting and ear disease.

Phytopathogenic fungus *Helminthosporium teres* showed the highest sensitivity to nano-preparation (demonstrated in Fig. 2). The inhibitory effects of nano-metals on the fungi colonies are summarized in Additional file 1: Table S1. The exception was the phytopathogenic fungus *Fusarium sp.*, which proved to be resistant to metal nano-particles. The maximum inhibition effect was found when *Helminthosporium teres* was grown on a medium with cobalt and silver nano-particles. In these conditions, the diameter of the colonies decreased by 27–53% and 36–53%, respectively. Other nano-metals also inhibited the growth of the fungus, but not more than 36%. A statistically significant decrease in the development of the phytopathogenic fungus *Alternaria sp.*

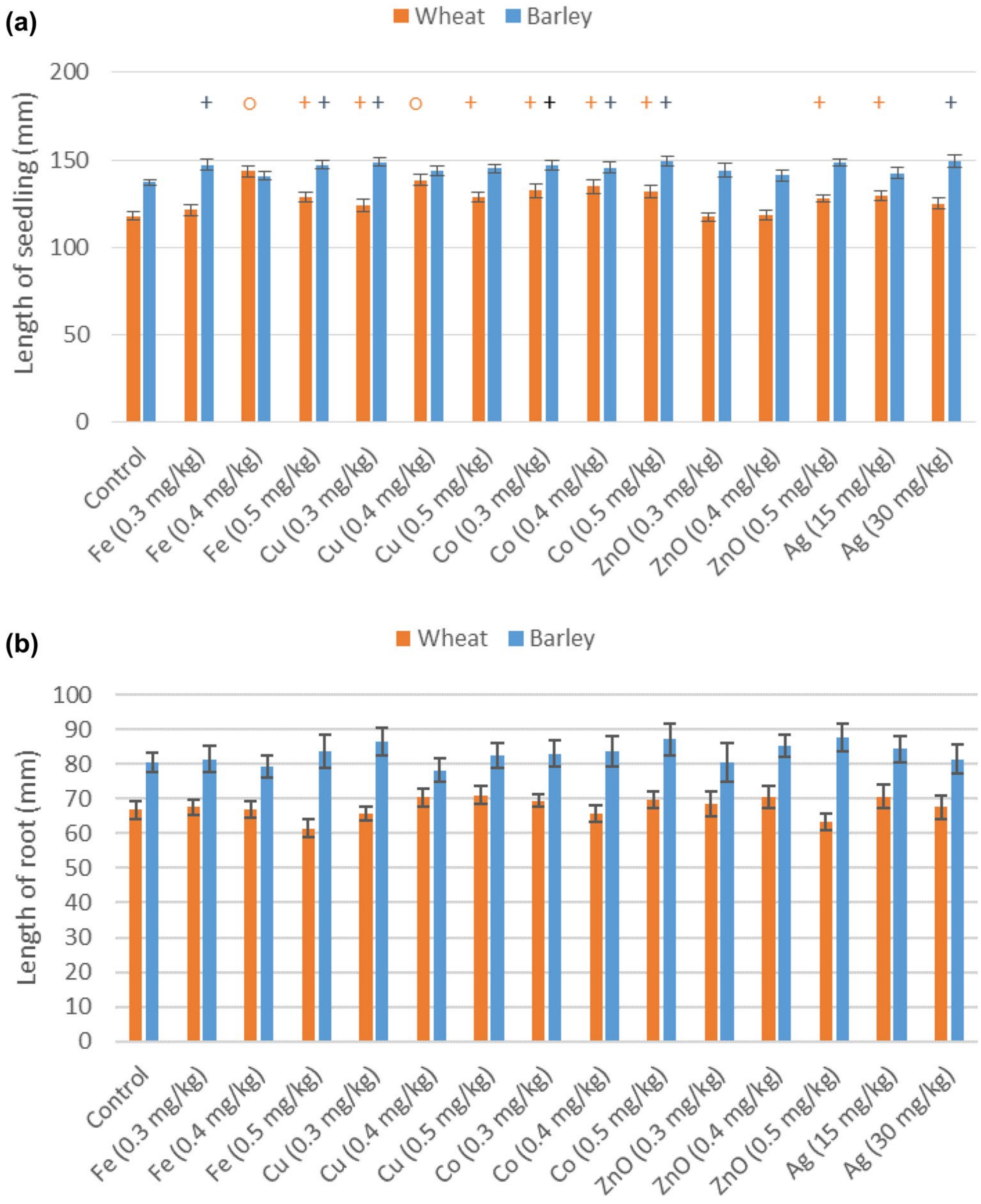


Fig. 1 Effect of metal nano-particles on the primary growth of seedlings **a** length of seedlings, **b** length of roots ("+" indicates 95% statistically significant difference to the control sample, "○" indicates 99% statistically significant difference to the control sample)

Fig. 2 Inhibition of mycelium growth of the phytopathogenic fungus *Helminthosporium teres* by the nano-particles Ag and Co

was observed when using nano-particles of chitosan-Ag (at 15 and 30 mg/kg), Co^0 (at 0.4 and 0.5 mg/kg), Cu^0 and ZnO (both at 0.5 mg/kg). These nano-metals slowed pathogen development by 27–33% relative to the control sample (Additional file 1: Table S1). These reductions were statistically significant at a 5% level.

As demonstrated in Fig. 3, the infection rate of untreated cereal grains was 44% for barley and 28% for wheat. Pre-sowing treatment of wheat grains with metal nano-particles in medium and high concentrations reduced the infection by 1.75–2 times. A more substantial suppression of phytopathogenic microflora was observed on barley. The number of infected grains decreased by 1.8–3.6 times in all experimental conditions. The highest reduction was obtained by silver particles. The exception was Fe^0 nano-particles, which did not have a significant impact on the seed infection. Thus, a distinguishable impact of metal nano-particles on seed infection was revealed. Therefore, nano-metals did not directly influence fungal spores but activated the protective mechanisms of plant cells. It was noted that metal nano-particles were particularly effective against the three fungi *Penicillium*, *Aspergillus* and *Mucor*, with suppression rates between 20 and 25%.

Influence of metal nano-particles on cell membranes and photosynthetic pigments

The barley/*Helminthosporium teres* was selected to investigate the impact of particles on cell membranes and photosynthetic pigments [26]. It was revealed that a pre-sowing treatment had a substantial effect on the lipid peroxidation products and water-soluble substances from plant tissues (Additional file 1: Table S2). Compared with untreated plants, the lipid peroxidation product was decreased by 10–40%, and water-soluble substances were decreased by 10–50%. It should be noted that low concentrations of nano-metals had a stronger impact on healthy plants. On the other hand, higher concentrations were more effective for infected seedlings. Amongst the particles, the most significant effects on the integrity of the cell walls were observed with zinc oxide and silver. The results are consistent with the anti-bacterial properties of silver particles [10].

Experimental results on the photosynthetic pigment complex [27] were also quantified (tabulated in Additional file 1: Table S3). For the uninfected barley plants, nanoparticles of Fe (0.25 mg/kg), Ag (0.5 mg/kg) and Cu (0.25 mg/kg) increased the content of total chlorophylls and carotenoids by 20% to 30%. In other cases, the number of photosynthetic pigments remained at the control level. During inoculation of barley seedlings

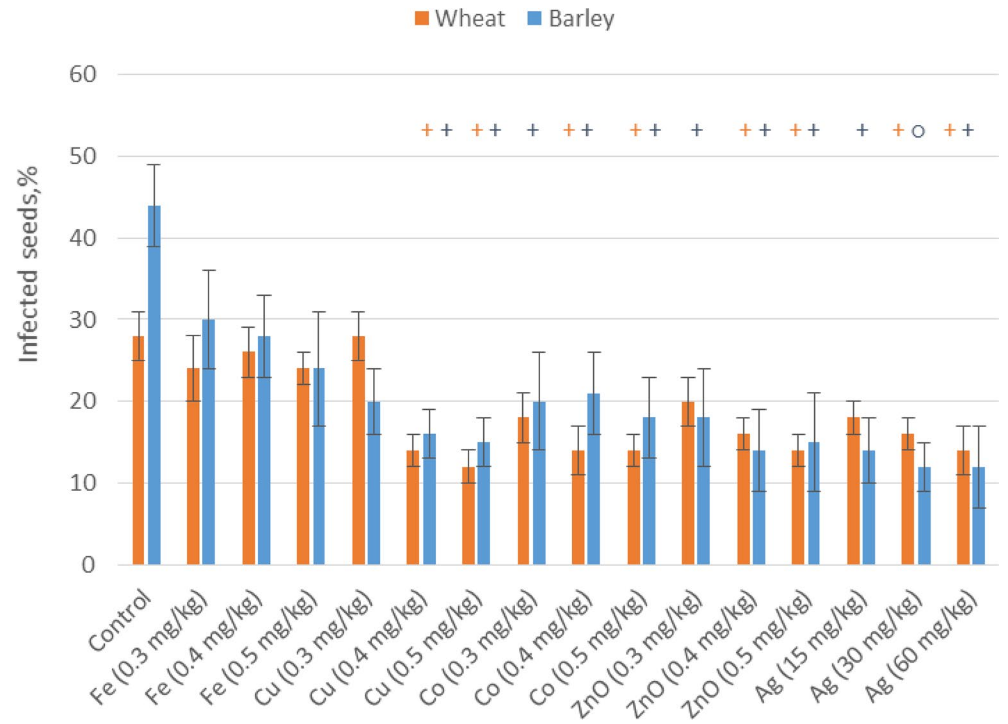


Fig. 3 The effect of metal nano-particles on the development of mycelium of phytopathogenic fungi (10th day). ("+" indicates 95% statistically significant difference to the control sample, "○" indicates 99% statistically significant difference to the control sample)

with *Helminthosporium teres* spores, a decrease in the content of chlorophylls (23%) and carotenoids (20%) in plant material was observed. The metal treatment significantly diminished the impact of the infection. In the cases of Fe (at concentrations of 0.25 and 0.5 mg/kg); Cu (at 0.5 mg/kg); ZnO (at 0.5 mg/kg) and Ag (0.5 mg/kg), the metal treatment completely overcome the infection.

Influence of pre-treatment on the productivity and the crop structure indicators

The results of studies on the effect of pre-sowing treatment of grains on crop productivity were measured in terms of stem length, numbers of productive and unproductive stems on plants and mass of straw. The full results for wheat and barley are presented in Additional file 1: Tables S4 and S5, respectively. The studies revealed that the stem length of wheat plants was influenced only by pre-treatment with iron nanoparticles at a concentration of 0.50 mg/kg grains. At this treatment, the height of plants increased by 5 cm compared to the control sample. In other conditions, the differences with control values were statistically insignificant. It is also noted that iron nanoparticles increased the number of productive stems in wheat plants by 1.3 times.

In contrast, the most noticeable improvement was observed in barley in straw mass (Additional file 1: Table S5). However, only iron and cobalt nano-particles (at both 0.25 and 0.5 mg/kg) increased the straw mass. In contrast, the samples treated with Cu^0 nanoparticles did not improve strawweight compared to the untreated plants.

Influences of pre-sowing treatment on the critical crop structure indicators are presented in Fig. 4. The experimental results showed an increase in the weight of ears from 10 plants by 1.2–1.4 times. The exception was the treatment of wheat grains with a high concentration of copper nanoparticles, where this indicator remained at the control level. However, it should be noted that the increase in ear mass from 10 wheat plants with the use of Cu nanoparticles in a concentration of 0.25 mg/kg of grains is solely due to an increase in the number of productive stems. In addition, an increase in the mass of the ear itself was revealed in other treatments. For example, with cobalt and iron nanoparticles, the mass of grains per ear increased by increasing the number of grains in the ear.

In comparison, the weight of 1000 grains remains practically unchanged in all variants and does not differ significantly from the control. Finally, it was noted that the pre-sowing treatment of barley grains with iron and cobalt nanoparticles at a concentration of 0.50 mg/kg of grains contributed to an increased weight of ears from 10 plants by 1.2–1.5 times (Fig. 4b). The increment was not attributed to the number of grains in the ear (Fig. 4d), but the grains size (Fig. 4e).

Conclusions

A moderate positive effect of seed pre-sowing treatment with aqueous dispersions of nano-particles zero-valence Fe^0, Cu^0, Co^0, ZnO and chitosan-Ag solutions on the growth of cereal seedlings were observed. The impact varied with the particle concentration. At medium and high concentrations of nano-metals Fe^0, Cu^0, Co^0, the length of the above-ground part of the wheat seedlings was increased by 14–22%. Nano-particles of ZnO and solution of chitosan-Ag had weaker, only from 6 to 10%. The impact on barley seedlings was weaker. The height of the above-ground part of barley seedlings increased by 6–9% compared to the control values. The application doses were very small, 0.5 mg/kg of grains. Most of the metal content is built up in the surface to the seedling shells and not the crop product as in the soil application [28].

A visible antifungal effect of metal nano-particles on phytopathogenic fungi was observed. The infection rate of untreated cereal grains was 44% for barley and 28% for wheat. Pre-sowing treatment of wheat grains with metal particles reduced the number of infected grains by 1.75–2 times. More potent suppression of the development of phytopathogenic microflora was observed on barley grains. The number of infected grains decreased by 1.8–3.6 times in all experimental conditions. The treatment also increased the synthesis of photosynthetic pigments and inhibited membrane lipid peroxidation. The decreased contents of water-soluble substances from plant tissues indicate an adaptive-protective mechanism. The analysis of the crop structure showed that the pre-treatment of grains with nanoparticles had substantial effects on stems and grains. The most significant improvement was observed with Fe^0 and Co^0 nano-particles. These nano-metals increased the average mass of stems by 20–60% and ear mass by 20–50%.

For all studied materials, the application of Co^0 nano-particles at 0.5 mg/kg produced the most significant improvement. The results can provide a promising economical and environmentally friendly pre-sowing treatment with ultra-low concentrations of metal nanoparticles.

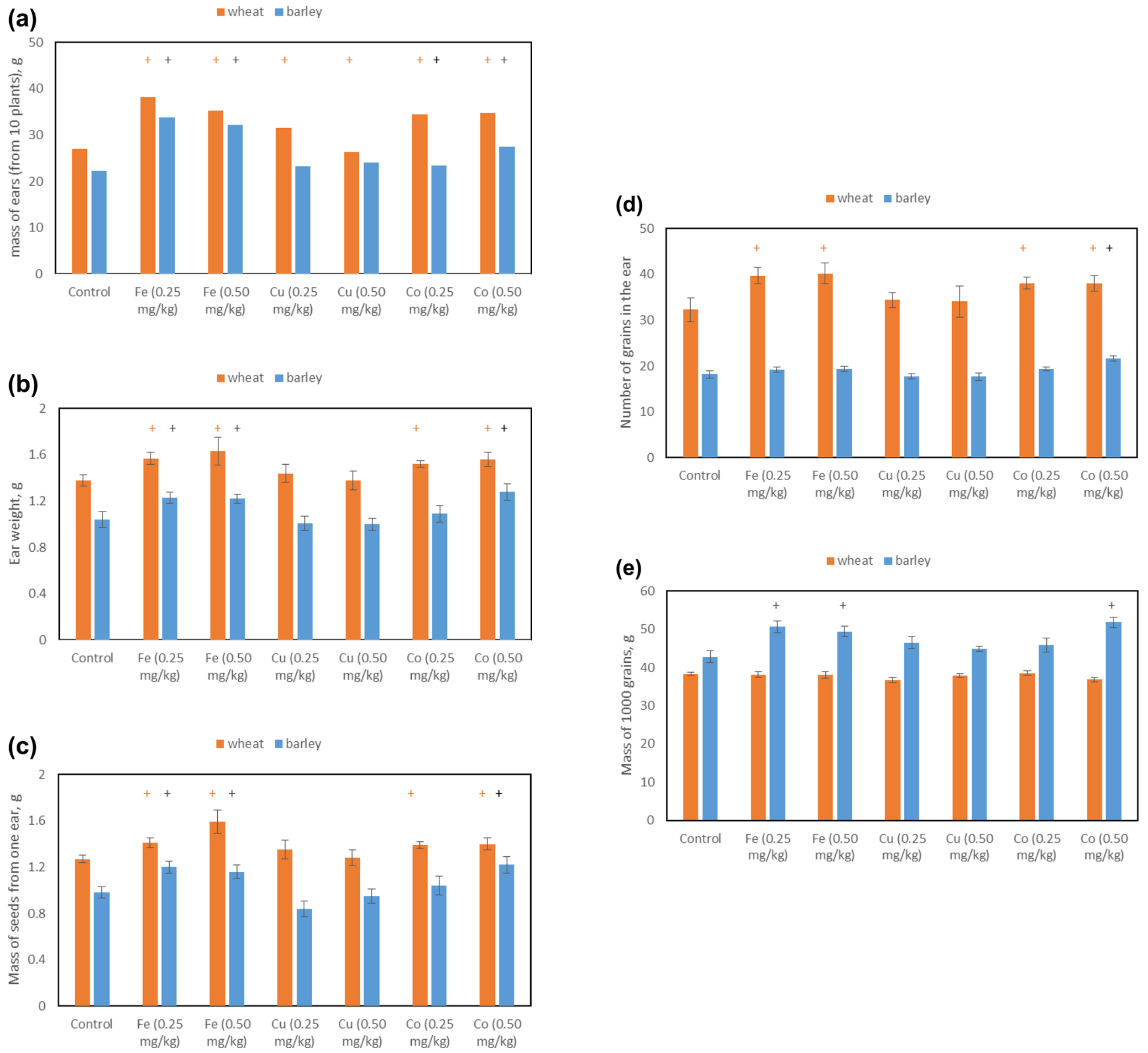


Fig. 4 Influence of pre-sowing treatment on the crop structure indicators: **a** mass of ears from 10 plants; **b** average ear weight; **c** mass of grains per ear; **d** number of grains per ear, and **e** mass of 1000 grains. ("+" indicates 95% statistically significant difference to the control sample, "○" indicates 99% statistically significant difference to the control sample). Other crop indicators are in Additional file 1: Tables S4 and S5

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-021-00277-w.

Additional file 1: Figure S1. SEM image (a) and XRD pattern (b) of Cu0 nanoparticles synthesized at 400°C. **Figure S2**. SEM image (a) and XRD pattern (b) of Fe0 nanoparticles synthesized at 400°C. **Figure S3**. SEM image of Co° nanoparticles with average size of 50 nm (a) and XRD pattern (b) of Co0 nanoparticles synthesized at 300°C. **Figure S4**. FE-SEM image (a) and size distribution (b) of Ag nano-particles. **Figure S5**. UV-Vis spectra of sample A500-C120 (AgNO3 500 ppm and chitosan 120 ppm). **Table S1**. The effect of metal nanoparticles on the development of mycelium of phytopathogenic fungi (10th day). **Table S2**. Effects of metal nanoparticles on barley plant cell membranes. **Table S3**. Effect of metal nanoparticles on photosynthetic pigments in barley seedlings. **Table S4**. Influence of pre-treatment of spring wheat seeds on the productivity indicators. **Table S5**. Influence of pre-treatment of barley seeds on the productivity indicators

Acknowledgements
Not applicable

Authors' contributions
CH, SH, KP, HN: synthesized the metal nano-particles. SP, KA, PN, MV: reagents and analytic tools, performed research and coordinated the field application and collected the data. SH, SP and CP: analysed the data and prepared the manuscript with inputs from all authors.

Funding
The studies were funded by projects coded QTBY02.02/19–20 of Vietnam Academy Science and Technology and BRFFR (№ B19VA-001) of Belarus's National academy of sciences.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 10 September 2021 Accepted: 26 November 2021

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