Evaluating Positive and Negative Effects of Seven Biogenic Metal-based Nanoparticles on Seed Germination and Seedling of Nano-primed Wheat and Flax Seeds

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Abstract

Seed germination is the first and the most susceptible stage in plant's growing phases, so could be considered as an index to evaluate the effect of newly developed materials like nanoparticles (NPs), providing useful information for researchers. In our experiments, germination tests have been carried out in Petri plates, containing wet filter paper and nano-primed seeds. We had biosynthesized seven nanoparticles in our previous researches, including calcinated and non-calcinated zinc oxide, zinc, magnesium oxide, silver, copper and iron nanoparticles. The effect of these biogenic nanoparticles and their counterpart metallic salts including zinc acetate, magnesium sulphate, silver nitrate, copper sulphate and iron (III) chloride was studied on two popularly grown plants, wheat and flax, in laboratory condition to obtain preliminary information for future field experiments. Germination percentage, shoot length, root length, seedlings length, root-shoot ratio, seedling vigor index (SVI), shoot length stress tolerance index (SLSI) and root length stress tolerance index (RLSI) were calculated at 2nd and 7th days of the experiment. According to the results, the response of the plants to metal containing nanoparticles and metal salts mainly depend on type of the metal, plant species, concentration of the NP suspension or salt solution, condition of the exposure and the stage of growth.

Introduction

The rapidly growing population and crop consumption cause a high demand of using fertilizers, which are critical for plant growth and improving the crop yield. In general, conventional mineral fertilizers are soluble salts, which easily dissolve in the soil media for plant uptake. However, a large portion of these soluble salts leach to the water resources, resulting in eutrophication and nutrient loss in fertilization. Solid forms of insoluble fertilizers also have been applied but the micronutrients are not so free to be easily bioavailable and easily transport to the water resources. Although, when the plants are in need, these solid minerals are less effective in supplying micronutrients in time due to their large size. To solve these problems, application of nanoparticles could be helpful hypothetically in both providing the micronutrients, minimizing the environmental contamination risks of soluble fertilizers and the problem of less bioavailability of solid fertilizers which is more environmentally benign (Singh et al. 2018; Liu et al. 2014). As the dimensions of materials reduce from a large size below 100 nm, significant changes in characteristics can occur mainly due to an increase in relative surface area (per unit mass) and then an enhanced chemical reactivity (Al-Hakkani 2020).

In recent years, a large number of reports have analyzed the influence of various nanostructures on different crops especially on their early developmental stage, seed germination and seedling development. Seed germination, i.e., the emergence of the radicle and primary root's elongation, is considered as the most sensitive stage of a plant life cycle. Priming with nanoparticle (nano-priming) could lead to positive, negative, or no impacts on germination process, depending on NPs type, size, concentration, duration of the exposure, or the growing conditions. Exact outcomes of the seed priming also consider as a technique to control the hydrate content of the seeds, stimulating metabolic activities for germination (Salah et al. 2015; Szollosi et al. 2020). In priming with NPs, first, the NPs have to penetrate the sclereids barrier of seed coat. Several studies revealed that NPs reach to the plant cells by crossing the intercellular spaces, bounding to a carrier protein, through aquaporin, ion channels, bounding to organic materials or endocytosis via making new pores. The NP-plant interaction may result in morphological and physiological changes, depending on characteristics of NPs (Singh et al. 2018; Aslani et al. 2014). It is also confirmed that some metal-based NPs can cross the seed coat and stimulate the embryonic differentiation through inducing the enzymes which interrupt seed dormancy. NPs may translocate to the other plant parts and cause various structural or functional changes in those parts (Alam et al. 2015). Previous reports also suggested different biochemical mechanisms for positive effect of nanomaterials upon NP exposure such as increased water uptake, remodeling of membrane lipids in seeds enhanced sugar metabolism and energy production, and the stimulated antioxidant defense (Szollosi et al. 2020). The impact of NPs on seed germination is related to their capability to enter the embryonic tissues through the seed coat. This capability is mostly related to the structure of seed coat and differs upon each plant species, physical and chemical characteristics of the ambience (Ko et al. 2017).

There is also another reason for evaluation of NP-plant interactions. Up to now, the amount of NPs existed in the environment is really lower than their toxic concentration. However, as NPs are widely commercialized, the potential biological impacts of NPs should be carefully assessed. As NPs have the potential to find their pathway into the environment and plants continuously interact with soil, water and air, these NPs may penetrate and translocate into the plant (Yang et al. 2017; Rastogi et al. 2017). In this regard, there will be a need to study the impact of NPs on plants.

Metals generally affect seed germination process, biochemical and physiological profiles and plant growth. Essential metals such as Zn, Mg, Cu and Fe are crucial for living cells and their deficiency could lead to damages in cell wall and DNA. Nevertheless, the excessive amounts of these metals or presence of non-essential metals (e.g., Ag) could be toxic due to causing oxidative stress, stimulating loss of membrane integrity, and injuring to proteins and DNA in a phytotoxic manner (Rai et al. 2017).

The objective of this study encompasses assessment potential impact of seven metal-based nanoparticles, which we have biosynthesized, characterized and applied in our previous works (Bayat et al. 2021a; Bayat et al. 2021b; Bayat et al. 2019), on seedling and seedling growth of two popular plants of wheat and flax though a laboratory study. Calcinated zinc oxide (C-ZnO), non-calcinated zinc oxide (NC-ZnO), zinc (Zn), magnesium oxide (MgO), copper (Cu) and iron (Fe) include essential metals which are vital for plant growth. Ag NP is selected due to its extensive use in industry and it could reach to the plants by surface water. Essential and nonessential elements might be absorbed by plant and according to their concentration, may result in toxicity. The effect of NPs also studied in some earlier reports. These nanoparticles showed both promoting and inhibition effects on plants growth (Vanninia et al. 2014; Asanova et al. 2019; Gorczyca et al. 2018). Moreover, the influence of metal salts, which are used as the precursors of the NPs during their biosynthesis, is compared with their counterpart NPs at the same concentrations. To the best of our knowledge, this report is the first to compare the positive and negative effects of these NPs on seedling parameters.

Materials And Methods

Biosynthesis of NPs

The plant extraction method, synthesis and characterization of applied NPs are described in our previous works (Bayat et al. 2021a; Bayat et al. 2019). In summary, dried strawberry leaves boiled in distilled water, filtered and mixed with NPs precursors. NPs generated by reduction of 0.01M precursor salt's solutions under heating, continuous stirring and addition of the extract drop by drop. Produced NPs washed with distilled water after centrifuge and dried at room temperature. C-ZnO and MgO NPs calcinated in furnace at 500ºC for 4h. The biosynthesized NPs specified using different characterization techniques including UV-Vis Spectroscopy, XRD, FESEM, EDS, Photon Cross-Correlation Spectroscopy (PCCS) and FT-IR. Biosynthesized NPs, their counterpart precursors, their average sizes and shapes are listed in Table1.

TABLE 1: list of the applied biogenic NPs, their counterpart precursors, the average size and shape of the NPs

NP	C-ZnO	NC-ZnO	Zn	MgO	Ag	Cu	Fe
Precursor	$Zn(CH2COO)_2$	$Zn(CH2COO)_2$	$Zn(CH2COO)_2$	$MgSO_4$	$AgNO_3$	$CuSO_4$	$FeCl_3$
Ave NP size	40	25	100	65	50	180×30	130×20
Shape	Spherical	spherical	Small sheets	Semi-spherical	spherical	sheets	Small sheets

Preparation of priming solutions

Different concentrations (50, 100, 150 ppm) of NPs (C-ZnO, NC-ZnO, Zn, MgO, $AgNO_3$, Cu and Fe) and also their counterpart metal salts ($Zn(CH2COO)_2$, $MgSO_4$, $AgNO_3$, $CuSO_4$ and $FeCl_3$) prepared in distilled water and the NPs dispersed by ultrasonic vibrations for 20 minutes. All dilutions were freshly prepared before use.

Preparation of seeds

Seeds of wheat (*Triticum aestivum L.*) variety Firuza 40 and flax (*Linum usitatissimum*) variety Semi Lini were used in this experiment. Each treatment consisted 30 randomly selected seeds with three replications. Seeds were kept in dry place at room temperature prior to use.

(a) Preparation of wheat seeds: Viability of seeds checked visually and then by suspending in distilled water, discarding the seeds floating above were and selecting seeds settled at bottom of the water for further experiment. Seeds immersed in a 5% sodium hypochlorite solution and rinsed with distilled water after 10 minutes, for surface sterility of the seeds (USEPA 1996). Then seeds soaked in a prepared NPs suspensions or metal salt solution for 12 hours. A set of seeds was soaked in distilled water without providing any treatment as control.

(b) Preparation of flax seeds: Seeds were checked visually for removing damaged seeds from the samples. Then seeds soaked in NPs suspensions or metal salt solutions for 12 hours. A set of seeds was used without providing any treatment as a control and soaked in distilled water.

In vitro germination of seeds

One piece of filter paper was placed into a Petri plate (10 cm in diameter), and for wetting the paper, 5 ml distilled water was added using a Pasteur pipette. Then 30 nano-primed seeds were transferred onto each filter paper. The Petri plates incubated at room temperature for seven days.

Measurement of physiological indexes

Germination percentages, shoot length, root length, seedlings length, root-shoot ratio, seedling vigor index (SVI), shoot length stress tolerance index (SLSI) and root length stress tolerance index (RLSI) were calculated at 2^{nd} and 7^{th} days. Means and standard deviations were derived from measurements on three replicates for each treatment and controls. A seed was considered germinated after the emergence of radicles or plumules from the seed coat (Ahmed et al., 2019). The length of roots and shoots were measured using a ruler with centimeter and millimeter scale (fig. 1).

(a) Shoot and root length: At 2^{nd} and 7^{th} days of the experiment, 10 seedlings from petri plate randomly selected to measure shoot and root lengths (Rawat et al. 2018).

(b) Seedling length: is considered as the sum of shoot length and root length of a seed (Rawat et al. 2018).

(c) Germination percentage: The germination percentage was calculated based on the total number of germinated seeds at the day of the experiment. Germination percentage calculated using the following equation (Raskar et al. 2013):

Germination percentage (%) = (average number of germinated seeds/total number of seeds) × 100

(d) Root/Shoot Ratio: The root to shoot ratio for each seedling was calculated as follows (Raskar et al. 2013):

Root/Shoot Ratio = average root length/ average shoot length

(e) Seedling Vigor Index (SVI): The seedling vigor index was computed by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed as an index number (Ushahra et al. 2013):

Seedling Vigor Index (SVI) = [average root length (cm)+average shoot length (cm)] × average germination percentage

(f) Shoot length stress tolerance index (SLSI) calculated using following equation (Ahmed et al., 2019; Raskar et al., 2013):

SLSI (%) =average shoot length of treated seedlings/average shoot length of control seedlings ×100

(g) Root length stress tolerance index (RLSI) calculated as follow (Ahmed et al. 2019; Raskar et al. 2013):

RLSI (%) =average root length of treated seedlings/average root length of control seedlings ×100

Statistical analysis

The obtained data statistically analyzed using Microsoft Excel software (version 2019), SAS and MSTAT-C statistical programs. One-way analysis of variance (ANOVA) applied for performing statistical analysis and p-value <0.05 considered as significant. Mean comparison performed by Least Significant Different (LSD) test. Means and standard deviations obtained from measurements on three replicates for control and each treatment.

Results

In the present research, we carried out experiments to compare the effect of our synthesized biogenic NPs (C-ZnO, NC-ZnO, Zn, MgO, Ag, Cu and Fe) and their counterpart metallic salts ($Zn(CH_3COO)_2$, $MgSO_4$, $AgNO_3$, $CuSO_4$ and $FeCl_3$) on germination and seedling growth of wheat and flax by considering their effect on different parameters including germination percentage, shoot length, root length, seedlings length, root/shoot ratio, seedling vigor index (SVI), shoot length stress tolerance index (SLSI) and root length stress tolerance index (RLSI). Three different concentrations of 50, 100 and 150ppm were used for seed priming. These concentrations were selected considering previous reports (Gorczyca et al. 2018; Younes et al. 2020). Wheat and flax plantlets were grown *In vitro* and all the observations were recorded up to 7 days. Results are tabulated in Tables 2 to 9. We observed that plant growth parameters of wheat and flax, varied considerably among the plants and also different priming solutions with various concentrations. Moreover, both effects of “stimulation” and “phytotoxicity” and in some cases no significant effect of NPs and their counterpart salts was observed on germination and seedling growth. The p-values less than 0.05 indicate there is a significant difference within the results.

Effect of biogenic NPs and their counterpart salts on physiological characteristics of wheat seedling

Tables 2-5 summarize the effect of priming with biogenic NPs and their counterpart metal salts (precursors) on seed germination parameters of wheat on 2^{nd} and 7^{th} day.

Germination percentage

The data showed that exposure to different concentrations of biogenic NPs resulted in an increase in germination percentage (G%) of all the treatments over the control at 2^{nd} day (Table 2). The maximum G% is related to the seeds primed with Zn NP at concentration of 150 and then 100ppm i.e., 98 and 96% respectively. Vise a versa, a decrease in G% occurred in samples treated with metal salts, comparing to the control, except the sample primed with 50ppm of zinc acetate in which G% was similar to the control (Table 4).

At 7^{th} day, an increase was observed in most of the samples and the highest G% was related to the concentrations of 50 and 150ppm of Zn NPs and also 50ppm of Cu NPs treated seeds (Table 3). Interestingly, for the samples treated with metal salts, the G% values are similar or close to the control except to the seeds primed with iron chloride, which showed a concentration dependent increase (Table 5). Among all of the applied NPs, Zn NPs found to be more effective in developing the seed germination of wheat seeds.

Shoot length and SLSI

At 2^{nd} day of the experiment, the best results of shoot elongation were related to the seeds treated with NC-ZnO NPs among all of the used NPs (Table 2) and also the maximum SLSI caused by 50 and 100ppm of NC-ZnO and 50ppm of Fe NPs treatments (about 115 to 120%). In the case of priming with salts (Table 4), the shoot elongations and SLSI values were less than the control, except 100ppm zinc acetate which was close to the control. Ag NPs induced a significant decrease in shoot length and SLSI, both in 2^{nd} and 7^{th} day. According to the Tables 3 and 5, the maximum shoot lengths and SLSI at 7^{th} day are related to the concentrations of 50ppm of Zn and Fe, and also 50 and 150ppm of Cu NPs (108 to 112%). In salt primed samples, just priming with 100ppm of $FeCl_3$ had a positive effect on SLSI (125%) and no other significant increase was observed. There were also decrease of shoot length in $CuSO_4$ primed samples.

Root length and RLSI

According to the Tables 2&4, at the 2^{nd} day of the test, the most significant promoting effect on root length and RLSI is related to the soaking with NC-ZnO NPs. Root lengths of the Zn NPs treated seeds were similar to the control and the other NP treatments had an inverse effect on root elongation. Considering the results of the priming with salts, the increase in root lengths and RLSI observed in zinc acetate treated seeds, also in 150ppm of $MgSO_4$ and $AgNO_3$ priming and also in 50ppm of $CuSO_4$ treatments. For seeds treated with $FeCl_3$, 50 ppm $MgSO_4$ and 100ppm $CuSO_4$, a significant decline was indicated.

In 7^{th} day (Table 3&5), there were a significant improvement in root length for all of the NP-primed seeds, except Ag NP-primed samples. NC-ZnO had the best effect on root length development and Ag NP showed a dose dependent inhibition effect on root lengths. For metal salt priming cases, the best results in root elongation and RLSI were related to the 100 and 150ppm of $FeCl_3$ and then 100 and 150ppm of zinc acetate priming. For the other samples there were a decrease in root length, mainly in $CuSO_4$ primed seeds.

Seedling length

In 2nd day (Tables 2&4), for Ag primed seeds, there were a remarkable decrease in seedling length. Soaking with 100&150ppm NC-ZnO and 50&150ppm Zn NPs exhibited an increase in seedling development. Additionally, zinc acetate was the most effective salt in improving seedling length and $CuSO_4$ was the most toxic one. For other samples the results were similar to the control or just a little lower than the control.

In 7th day (Tables 3&5), for all NP treatments we observed seedling length improvement in comparison with the control, except Ag NP treated seeds. The maximum effect obtained with 150ppm C-ZnO, 100ppm NC-ZnO and 150ppm MgO NPs (about 25% more than the control).

For metal salt primed seeds, application of $CuSO_4$, $AgNO_3$ and $MgSO_4$ led to a notable inhibition of seedling growth respectively. $FeCl_3$ treatments, had the best improving effect in a dose dependent manner.

Root/soot ratio (R/S)

Under specific conditions, higher proportion of roots can help plants to compete more efficiently for water uptake and soil resources, while a higher proportion of shoots can help plants to collect more light energy (Allaby, 2006). In 2nd day (Table 2&4), all of the samples had the R/S of more than 1, indicating root lengths longer than shoot lengths and the values were similar to the control or less than that, except the Ag NP treatments. In Ag NP primed seeds, a great improvement in R/S observed mainly at the concentration of 100ppm which was about 1.5 times more than its respective control and the minimum was related to the 50ppm Fe NP which was 0.3 times lower than the control. For metal salt treatments, $AgNO_3$ showed the results close to the Ag NP treatments, zinc acetate had the most effect in R/S values and $CuSO_4$ had a dose dependent decrease. Overall, the R/S in salt treated seeds was more than NP treated ones.

In 7th day, R/S ratios of the NP treated seeds are higher than the control, except 150ppm Ag treated one and the maximum was for 150ppm C-ZnO NP treatments (Table 3). For salt priming, the amounts are less than NP treated seeds and just 100&150ppm zinc acetate and 110&150ppm $FeCl_3$ root to shoot ratios were higher than the control (Table 5).

SVI

For evaluating the effect of metal NPs or metal salts on seedling growth, the seedling vigor index (SVI) could be used as a phytotoxicity index (Zhao et al. 2016). In 2nd day, Ag NP priming resulted in minimum values of SVI. NC-ZnO and Zn NPs had values higher than the control and in salt treated seeds SVIs were less than NP treated seeds. The minimum amounts are due to the $CuSO_4$ and $FeCl_3$ treatments. In 7th day, for NP treatments, all SVI amounts were considerably more than the control. The maximum SVI observed in 150ppm of C-ZnO, 50&150ppm of Zn NP and 150ppm of MgO NP primed samples. For salt treatments, 100&150ppm of zinc acetate and 150ppm of $FeCl_3$ solutions had the maximums SVIs. Besides, $CuSO_4$ and $AgNO_3$ showed the most inhibition effect.

TABLE 2 effect of biogenic NPs priming on seed germination parameters of wheat on 2nd day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Nanoparticle	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RLSI
C-ZNO	0 (Control)	83.33±3.33e	2.64±0.27b	4.26±0.27ab	6.90±0.45ab	1.62±0.16c	574.97±37c	100.00±0.00b	100.0
	50	90.00±1.61bc	2.36±0.28c	3.12±0.41c	5.48±0.56d	1.33±0.20d	493.20±51de	89.85±11.60cd	73.67
	100	86.66±2.52cd	2.30±0.30c	3.08±0.29c	5.38±0.51de	1.35±0.19d	466.23±44e	87.10±8.21d	72.40
	150	93.33±1.87b	2.70±0.18b	4.34±0.67ab	7.04±0.77ab	1.61±0.23c	657.04±71b	102.76±7.94b	102.0
NC-ZnO	0 (Control)	83.33±3.33e	2.64±0.27b	4.26±0.27ab	6.90±0.45ab	1.62±0.16c	574.97±37c	100.00±0.00b	100.0
	50	93.33±2.11b	3.18±0.24a	4.86±0.54a	6.82±0.75b	1.53±0.11cd	750.37±70a	120.31±13.3b	115.0
	100	93.33±2.37b	3.08±0.21a	4.70±0.56a	7.78±0.72a	1.52±0.14cd	726.11±67a	117.16±8.30a	110.5
	150	86.66±1.26cd	2.84±0.26ab	4.96±0.37a	7.80±0.56a	1.75±0.15bc	675.94±48b	107.57±9.87b	116.4
Zn	0 (Control)	83.33±3.13e	2.64±0.27b	4.26±0.11ab	6.90±0.45ab	1.62±0.16c	574.97±37c	100.00±0.00b	100.0
	50	86.66±3.02cd	2.70±0.34b	4.50±0.79ab	7.20±0.93a	1.68±0.34c	623.95±80b	102.27±12.84b	105.6
	100	96.66±1.91ab	2.50±0.24bc	4.42±0.35ab	6.92±0.19ab	1.79±0.32bc	668.88±18b	94.69±9.27c	103.7
	150	98.88±2.23a	2.72±0.21b	4.56±0.38ab	7.28±0.46a	1.43±0.19d	719.84±46a	103.03±8.21b	107.0
MgO	0 (Control)	83.23±3.31e	2.64±0.27b	4.26±0.12ab	6.90±0.45ab	1.62±0.16c	574.97±37c	100.00±0.00b	100.0
	50	90.00±1.93bc	2.44±0.39bc	3.84±0.35b	6.28±0.71bc	1.59±0.16c	565.20±64cd	92.42±14.81c	90.14
	100	93.33±4.05b	2.46±0.30bc	3.46±0.23bc	5.92±0.32c	1.43±0.23d	552.51±30cd	93.18±11.55c	81.22
	150	93.31±3.21b	2.66±0.27b	3.82±0.69b	6.48±0.93bc	1.43±0.16d	604.78±87bc	100.75±10.23b	89.67
Ag	0 (Control)	85.00±2.90d	2.16±0.28cd	3.83±0.28b	6.00±0.50c	1.78±0.20bc	510.00±42d	100.00±0.00b	100.0
	50	87.00±1.07cd	1.60±0.41e	3.25±0.28bc	4.85±0.46f	2.14±0.61ab	421.95±40e	74.07±18.90e	84.85
	100	90.01±1.93bc	1.40±0.36ef	3.33±0.28bc	4.73±0.25f	2.53±0.88a	426.00±22e	64.81±16.69fg	87.03
	150	91.00±1.89bc	1.46±0.32ef	3.23±0.46bc	4.70±0.78f	2.23±0.20ab	427.70±71e	67.90±14.88f	84.42
Cu	0 (Control)	85.00±2.90d	2.16±0.28cd	3.83±0.28b	6.00±0.50c	1.78±0.20bc	510.00±42d	100.00±0.00b	100.0
	50	92.03±1.68b	2.20±0.10c	3.50±0.50bc	5.70±0.60d	1.58±0.15c	524.40±55d	101.85±4.62b	91.38
	100	87.06±2.07cd	2.30±0.21c	3.53±0.50bc	5.86±0.71cd	1.51±0.08cd	510.40±61d	108.02±9.63b	92.25
	150	90.01±1.93bc	2.00±0.50d	3.73±0.92b	5.73±1.41cd	1.86±0.11bc	516.00±127d	92.59±23.14c	97.47
Fe	0 (Control)	85.00±2.90d	2.16±0.28cd	3.83±0.28b	6.00±0.50c	1.78±0.20bc	510.00±42d	100.00±0.00b	100.0
	50	90.00±1.93bc	2.50±0.43bc	3.10±0.17c	5.6±0.40d	1.26±0.23de	504.00±36d	115.74±20.18a	80.93
	100	89.00±3.05c	1.87±0.49de	2.92±1.1cd	4.8±1.53f	1.54±0.39cd	427.20±136	86.80±22.79d	76.37
	150	90.01±1.93bc	2.10±0.26cd	3.40±0.79bc	5.5±1.01d	1.61±0.27c	495.00±91de	97.22±12.24c	88.77
p value	-	0.0050	0.0011	0.0002	0.0059	0.0010	0.0009	0.0016	0.000

TABLE 3 effect of biogenic NPs priming on seed germination parameters of wheat on 7th day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the p < 0.05 level.

Nanoparticle	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RLSI (
C-ZNO	0 (Control)	83.33±3.33cd	11.25±0.61bc	9.58±0.49f	20.83±0.9de	0.85±0.03d	1736±86g	100.00±0.00b	100.0
	50	90.00±1.26b	11.37±0.47bc	11.37±1.37d	22.75±0.9cd	1.00±0.15c	2047±86d	101.11±4.25b	118.6
	100	90.06±3.03b	11.12±0.81c	10.47±0.95e	22.5±0.5cd	1.05±0.16c	2026±51d	97.77±7.25bc	120.0
	150	93.33±2.54ab	11.25±0.85bc	14.25±0.95a	25.37±0.75a	1.29±0.17a	2368±69a	98.88±759bc	144.7
NC-ZnO	0 (Control)	83.33±3.33cd	11.25±0.61bc	9.58±0.49f	20.83±0.9de	0.85±0.03d	1736±86g	100.00±0.00b	100.0
	50	90.40±2.76b	11.25±1.04bc	13.37±1.10b	24.62±1.7ab	1.19±0.11b	2226±16b	100.00±9.25b	140.7
	100	90.03±1.73b	11.87±0.62b	13.37±0.75b	25.25±0.64a	1.13±0.11bc	2273±58b	105.55±5.59ab	139.5
	150	86.66±2.98c	11.60±0.41b	14.00±1.83a	24.60±1.7ab	1.20±0.17b	2218±15b	103.11±3.71ab	144.0
Zn	0 (Control)	83.33±3.33cd	11.25±0.61bc	9.58±0.49f	20.83±0.9de	0.85±0.03d	1736±86g	100.00±0.00b	100.0
	50	96.66±2.69a	12.25±2.72a	12.37±0.47c	24.62±2.6ab	1.04±0.21c	2380±25a	108.88±24.20a	129.1
	100	86.66±3.25c	11.37±1.10bc	12.62±0.47c	24.00±1.22b	1.11±0.12bc	2079±10cd	101.11±9.85b	131.7
	150	96.66±1.59a	11.75±0.64b	12.75±1.0bc	24.50±1.6ab	1.08±0.04c	2368±15a	104.44±5.73ab	133.0
MgO	0 (Control)	83.33±3.33cd	11.25±0.61bc	9.58±0.49f	20.83±0.9de	0.85±0.03d	1736±86g	100.00±0.00b	100.0
	50	93.33±1.87ab	11.47±0.05bc	12.12±0.85c	23.60±0.8bc	1.05±0.07c	2202±80b	102.00±0.44b	126.5
	100	93.33±3.23ab	10.87±0.47d	12.25±0.50c	23.12±0.47c	1.12±0.08bc	2158±44bc	96.66±4.25bc	127.8
	150	93.33±2.36ab	11.5±0.57c	13.37±1.37b	24.87±1.93a	1.16±0.06b	2321±18a	102.22±5.13b	139.5
Ag	0 (Control)	89.01±2.56bc	11.24±1.07bc	8.34±2.62g	19.58±3.18e	0.74±0.21e	1743±28g	100.00±0.00b	100.0
	50	89.5±2.98bc	10.82±0.91d	9.82±1.57ef	20.65±2.1de	0.90±0.13d	1848±18f	100.07±8.47b	100.0
	100	91.06±3.02b	9.92±0.90e	7.84±1.13gh	17.76±1.79f	0.79±0.09d	1617±16h	91.69±8.39c	79.85±
	150	92.33±3.11ab	9.36±0.84f	6.20±1.03i	15.57±1.61g	0.66±0.10ef	1438±149i	86.56±7.85cd	63.21±
Cu	0 (Control)	89.01±2.56bc	11.24±1.07bc	8.34±2.62g	19.58±3.18e	0.74±0.21e	1743±283g	100.00±0.00b	100.0
	50	95.34±2.44a	12.17±0.78a	10.89±1.1de	23.06±1.30c	0.89±0.11d	2052±11d	112.49±7.23a	110.91
	100	89.66±2.81bc	11.39±1.05bc	10.65±1.54e	22.04±2.04d	0.94±0.14d	1976±18de	105.30±9.74ab	108.4
	150	91.1±1.20b	11.93±0.90ab	12.43±1.13b	24.36±1.7ab	1.04±0.09c	2219±15b	110.32±8.37a	126.5
Fe	0 (Control)	89.01±2.56	11.24±1.07bc	8.34±2.62g	19.58±3.18e	0.74±0.21e	1743±28g	100.00±0.00b	100.0
	50	91.3±1.95b	11.98±0.58ab	11.14±0.78d	23.13±1.15c	0.93±0.06cd	2112±10c	110.78±5.43a	113.4
	100	91.85±3.03b	10.96±0.80cd	10.67±1.1de	21.64±1.58d	0.97±0.10cd	1987±14de	101.33±7.43b	108.7
	150	91.23±1.23b	11.32±1.40bc	10.63±1.0de	21.95±1.58d	0.95±0.15cd	2002±144d	104.62±12.9ab	108.2
P value		0.0100	0.0008	0.0066	0.0003	0.0040	0.0009	0.0160	0.003

TABLE 4 Effect of metal salt (precursors) priming on seed germination parameters of wheat on 2nd day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Metal salt	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RLSI (%)
$Zn(CH_3CO_2)_2$	0 (Control)	98.88±1.92a	1.78±0.64a	2.41±0.37cd	4.20±0.69bc	1.47±0.40de	415±6ab	100.00±0.00a	100.00±0
	50	98.88±1.92a	1.7±0.20a	3.00±0.33b	4.70±0.36b	1.78±0.30cd	464±35a	95.50±11.23ab	124.48±1
	100	93.33±2.65c	1.78±0.21a	3.35±0.27a	5.13±0.38a	1.89±0.23c	467±36a	100.18±12.00a	139.00±1
	150	96.66±2.92b	1.48±0.09b	2.81±0.54c	4.26±0.54bc	1.88±0.41c	412±5ab	83.33±5.52c	115.49±2
MgSO4	0 (Control)	98.88±1.92a	1.78±0.64a	2.41±0.37cd	4.20±0.69bc	1.47±0.40de	415±6ab	100.00±0.00a	100.00±0
	50	86.66±3.02e	1.25±0.16d	1.55±0.10e	2.80±0.22ef	1.25±0.14f	242±19d	70.22±9.23d	64.31±4.3
	100	93.33±2.65c	1.58±0.24ab	2.75±0.30c	4.33±0.18bc	1.78±0.40cd	404±12b	88.95±13.49bc	114.10±1
	150	96.66±1.92b	1.51±0.16b	3.16±0.25ab	4.68±0.38b	2.09±0.17b	452±36a	85.20±9.00bc	131.39±1
AgNO3	0 (Control)	98.88±1.92a	1.78±0.64a	2.41±0.37cd	4.20±0.69bc	1.47±0.40de	415±6ab	100.00±0.00a	100.00±0
	50	83.33±3.25f	1.38±0.20bc	2.91±0.20bc	4.30±0.24bc	2.16±0.45ab	358±20c	77.71±11.49cd	121.02±8
	100	90.12±2.98d	1.08±0.13d	2.38±0.37cd	3.46±0.34d	2.23±0.51a	312±31cd	60.86±7.46e	98.89±15
	150	96.66±2.92b	1.31±0.21bc	2.78±0.34c	4.10±0.4cc	2.16±0.41ab	396±40b	73.97±12.00d	115.49±1
CuSO4	0 (Control)	98.88±1.92a	1.78±0.64a	2.41±0.37cd	4.20±0.69bc	1.47±0.40de	415±6ab	100.00±0.00a	100.00±0
	50	90.89±3.12d	1.53±0.08bc	3.11±0.37b	4.65±0.37b	2.03±0.28b	422±3ab	86.14±4.58bc	129.32±1
	100	90.23±3.65d	1.31±0.29bc	1.63±0.48e	2.95±0.66e	1.26±0.33f	266±60d	73.97±16.44d	67.77±19
	150	96.66±2.92b	1.20±0.18d	1.45±0.19f	2.65±0.18fg	1.24±0.30f	256±1d	67.41±10.65de	60.16±8.1
FeCl3	0 (Control)	98.88±1.92a	1.78±0.64a	2.41±0.37cd	4.20±0.69cd	1.47±0.40de	415±6cd	100.00±0.00a	100.00±0
	50	90.62±3.02d	1.61±0.17a	1.69±0.10e	3.29±0.10d	1.25±0.13f	299±9d	90.90±6.56b	70.42±2.3
	100	94.83±3.03c	1.41±0.38b	1.58±0.50e	2.98±0.64e	1.61±0.44d	283±57d	79.54±14.42c	76.99±11
	150	98.33±2.65a	1.44±0.46b	2.07±0.49d	3.52±0.52d	1.81±0.67c	346±49c	81.06±17.48c	85.91±11
P value		0.0008	0.0111	0.0101	0.0022	0.0009	0.0100	0.0061	0.0004

TABLE 5 Effect of metal salt (precursors) priming on seed germination parameters of wheat on 7th day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Metal salt	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RLSI (%)
Zn	0 (Control)	98.88±1.92a	10.03±0.70b	8.99±0.84c	19.00±1.4d	0.89±0.07b	1881±141c	100.00±0.0b	100.00±0.
$(CH_3CO_2)_2$	50	98.66±2.10a	10.00±1.47b	8.26±1.67cd	18.26±2.41e	0.83±0.19c	1802±237c	99.70±14.71b	91.95±18.
	100	97.66±2.65a	10.32±0.82b	10.60±1.33b	20.92±1.96b	1.02±0.10a	2043±192a	102.90±8.2ab	114.81±9.
	150	98.88±1.92a	9.85±1.18bc	10.58±1.63b	20.43±2.4bc	1.07±0.14a	2020±242a	98.20±11.82b	117.72±1
MgSO4	0 (Control)	98.88±1.92a	10.03±0.70b	8.99±0.84c	19.03±1.43d	0.89±0.09b	1881±112c	100.00±0.0b	100.00±0.
	50	95.02±3.02bc	8.62±2.59cd	7.45±1.80d	16.07±4.30f	0.90±0.17b	1526±409e	85.95±25.8cd	82.86±20.
	100	98.33±2.25a	10.07±0.56b	8.73±1.45cd	18.80±1.5de	0.86±0.15bc	1843±155c	100.46±5.67b	98.09±16.
	150	98.88±1.92a	9.73±1.08c	8.48±1.92cd	18.21±2.86e	0.86±0.13bc	1639±257d	97.01±10.8bc	94.37±21.
AgNO3	0 (Control)	98.88±1.92a	10.03±0.70b	8.99±0.84c	19.01±1.41d	0.89±0.05b	1881±121c	100.00±0.0b	100.00±0.
	50	97.06±3.25ab	9.28±0.72c	7.10±1.45d	16.39±2.05f	0.76±0.11d	1591±199de	92.57±7.24c	79.05±16.
	100	97.03±2.98ab	7.70±1.07d	4.62±0.90e	12.33±1.45g	0.61±0.15e	1196±140ef	76.85±10.72d	51.44±10.
	150	98.88±1.92a	8.47±1.04cd	4.25±1.13e	12.72±1.88g	0.50±0.11f	1258±186e	84.49±10.4cd	47.27±12.
CuSO4	0 (Control)	98.88±1.92a	10.03±0.70b	8.99±0.84c	19.02±1.43d	0.89±0.04b	1881±141c	100.00±0.0b	100.00±0.
	50	97.06±3.12ab	7.73±2.77d	4.53±2.17e	12.27±4.7g	0.58±0.17e	1191±457ef	77.15±27.67d	50.48±24.
	100	96.66±2.92b	7.83±1.29d	1.87±0.74f	9.70±1.88h	0.23±0.07g	938±182g	78.09±12.87d	20.83±8.3
	150	98.88±1.92a	5.55±0.82e	1.22±0.65f	6.77±0.87i	0.23±0.14g	670±86h	55.33±8.27e	13.66±7.3
FeCl3	0 (Control)	98.88±1.92a	10.03±0.70b	8.99±0.84c	19.02±1.43d	0.89±0.04b	1881±141c	100.00±0.0b	100.00±0.
	50	86.66±2.96d	11.12±0.25a	8.25±2.17cd	19.37±1.93d	0.74±0.20d	1679±167d	98.88±2.22b	86.11±22.
	100	90.00±3.03cd	11.15±0.30a	10.75±1.84b	21.90±1.79b	0.96±0.17ab	1971±16ab	125.43±3.37a	112.21±1
	150	93.33±2.65c	11.12±0.47a	11.25±0.64a	22.37±0.62a	1.01±0.08a	2088±58a	98.88±4.15b	117.43±6.
P value		0.0200	0.0000	0.0055	0.0007	0.0108	0.0077	0.0100	0.0006

Effect of biogenic NPs and their counterpart salts on physiological characteristics of flax seedling

Tables 6-9 summarize the effect of priming with biogenic NPs and their counterpart metal salts (precursors) on seed germination parameters of flax on 2nd and 7th day.

Germination percentage

Considering the results of the Tables 6&8, in early stages of seedling, the maximum seed germination percentage (90%) was related to the flax seeds soaked with 100ppm C-ZnO NPs and all of the other treatments showed G% similar or less than the control. The minimum G% was related to the seeds soaked with 100ppm of Cu NPs. Among metal salt, zinc acetate had improving effect on G% and except 100ppm of $CuSO_4$ and $FeCl_3$ primed seeds, no increase in G% was observed with respect to the control. At 7th day of the experiment (Tables 7&9), just 150ppm of C-ZnO NPs suspension had improving effect over the control and all of the other NP treatments showed inhibition effect. Among salts, G% of the 150ppm of $MgSO_4$ treated seeds was close to the control and the others were less than the control. 150ppm of $AgNO_3$ had such a severe toxic effect so no gemination was observed and there are not any data reported in following for this case.

Shoot length and SLSI

According to the Tables 6&8, at 2nd day of the test, all of the NP treatments showed enhancement in shoot length over the control. The most effective NP was NC-ZnO and the less effective one was Ag. The most effective salts in improving shoot length were $MgSO_4$ and then zinc acetate. 50ppm of $AgNO_3$ was also very effective in this regard and 100ppm concentration of $AgNO_3$ and $CuSO_4$ had the worst effect on shoot elongation. Tables 7&9 record the results of the 7th day of the experiment which show that the most effective NP in shoot length development of flax seeds was Ag.

$MgSO_4$ and $FeCl_3$ were the most effective in shoot length development and 100ppm $AgNO_3$ was the most toxic one, completely inhibiting the germination of seeds.

Root length and RLSI

Flax seeds responded differently toward the treatment at various concentrations of NPs. At 2nd day (Table 6&8), Zn NPs had the best effect on root growth. Ag and Fe NPs had the most inhibition effect. $MgSO_4$ was the most effective salt in root length development and $CuSO_4$ was the most toxic one. At 7th day of the experiment, a significant increase in root length observed in most of the treatments, especially in Zn NPs treated samples and the root lengths were about 2 to 3 times higher than the control which is considered as a great positive effect. Cu 100ppm besides 50 and 150ppm concentrations of Fe NPs showed a notable toxic effect on root length parameter. Zinc acetate and $MgSO_4$ solutions induced a significant root length increase of about twofold over the control. 100 and 150ppm of $CuSO_4$ priming inhibited the root growth to the lengths about 27% of the control.

Seedling length

Like root and shoot length, the best results of seedling length is related to Zn NPs treated samples. After 48h (Tables 6&8), Ag and Fe had seedling lengths less than the control. For $AgNO_3$ primed samples, 50ppm concentration resulted in maximum seedling length and 100ppm resulted in no seedling. Moreover, $MgSO_4$ showed the best increasing effect in seedling length. After the first week (Tables 7&9), in the case of nano-primed seeds, except Cu and Fe primed samples which had seedling lengths less than the control, the other treatments had seedling lengths more than the control. Zinc acetate and $MgSO_4$ were most effective in increase of seedling length and the minimum was related to the 100ppm $AgNO_3$ treatments.

Root/Shoot ratio

In early stages of flax seedling, the shoot grew with a higher speed in comparison with root. Considering the results of the Tables 6&8, at 2nd day, all of the R/S values were less than the control in NP and salt primed samples. At 7th day, except Fe NPs and 100-150ppm Cu NPs primed seeds, the other samples had R/S more than the control. 100 and 150ppm of $CuSO_4$ (like Cu NP) and 100ppm of $FeCl_3$ treated seeds had R/S less than the control.

SVI

At 2nd day of the experiment, maximum SVI was related to the 150ppm C-ZnO and 50ppm of Zn NPs due to their high seedling length values and the minimum was related to the 100ppm of Cu NPs primed samples. At 7th day, Fe NPs primed samples had the minimum SVIs due to their minimum seedling lengths. $MgSO_4$ primed samples had the maximum SVIs and 50ppm zinc acetate and 100ppm $AgNO_3$ had the minimum SVIs.

TABLE 6 Effect of biogenic NPs priming on seed germination parameters of flax on 2nd day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Nanoparticle	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RL
Non(Control)	0	86.61±2.12b	0.32±0.04ef	2.24±0.28cd	2.56±0.28c	7.10±1.31a	221.84±24.96c	100.00±0.00f	10
C-ZnO	50	86.56±2.23b	0.47±0.04de	1.84±0.47e	2.32±0.49cd	3.83±0.87d	201.05±43.06cd	150.00±13.97e	82.
	100	90.00±1.93a	0.66±0.11bc	2.52±0.20c	3.18±0.25b	3.90±0.72d	286.20±23.29b	206.25±35.63b	11
	150	83.33±3.3bc	0.74±0.16b	3.12±0.41a	3.86±0.49a	4.35±1.02cd	321.65±41.07a	231.25±52.29ab	13
NC-ZnO	50	80.03±3.33c	0.64±0.18c	2.74±0.37bc	3.38±0.25b	4.73±1.99c	270.50±20.71b	200.00±56.78b	12
	100	80.11±2.98	0.84±0.15a	2.32±0.15cd	3.20±0.22b	2.87±0.50e	256.35±17.91bc	262.5±47.39a	10
	150	83.43±3.33c	0.82±0.10a	2.20±0.23cd	3.02±0.19b	2.73±0.55e	251.65±16.02bc	256.25±34.23a	98.
Zn	50	86.66±2.12b	0.74±0.25b	3.00±0.50ab	3.74±0.68a	4.33±1.22bc	324.10±58.96a	231.25±78.43ab	13
	100	73.43±4.01d	0.70±0.12b	2.50±0.60c	3.20±0.51b	3.78±1.54d	234.65±38.10c	218.75±38.27b	11
	150	73.33±4.04d	0.70±0.21b	3.08±0.40ab	3.78±0.59a	4.61±0.99c	277.18±43.50b	218.75±66.29b	13
MgO	50	76.82±3.15d	0.44±0.13de	2.30±0.62cd	2.74±0.71c	5.46±1.74b	210.04±54.63cd	137.50±41.92de	10
	100	80.00±2.45c	0.56±0.08d	2.30±0.27cd	2.86±0.25bc	4.20±0.88c	228.80±20.07c	175.00±27.5c-e	10
	150	73.33±3.25d	0.66±0.08bc	2.42±0.37c	3.08±0.37b	3.74±0.87d	225.85±27.14c	206.25±27.95b	10
Ag	50	83.43±3.20c	0.36±0.15ef	1.63±0.65ef	2.00±0.75de	4.65±1.31c	166.66±62.91e	114.58±47.73f	72.
	100	83.83±3.35c	0.44±0.13de	1.66±0.20ef	2.10±0.1d	4.52±3.06c	174.99±8.33de	137.50±41.92ef	74.
	150	80.24±2.86c	0.46±0.6c-e	1.42±0.35fg	1.88±0.44f	3.33±1.36de	150.85±31.85ef	143.75±52.29e	63.
Cu	50	86.66±2.52b	0.58±0.16cd	2.33±0.47cd	2.94±0.61bc	4.20±0.73c	254.78±52.92bc	181.25±51.34cd	10
	100	61.00±3.70e	0.38±0.16e	1.50±0.38f	1.88±0.46f	4.55±2.37c	114.68±28.41g	118.75±51.34f	66.
	150	76.55±4.31d	0.62±0.13c	1.58±0.40f	2.20±0.43cd	2.64±0.90ef	166.65±32.97e	193.75±40.16bc	70.
Fe	50	86.55±2.42b	0.42±0.17e	1.26±0.27h	1.86±0.29f	3.39±1.28d	145.58±25.56ef	131.25±55.90de	56.
	100	76.66±4.26d	0.50±0.14d	1.84±0.29e	2.34±0.28cd	3.95±1.40cd	179.38±22.08de	156.25±44.19e	82.
	150	86.66±2.52b	0.62±0.13c	1.58±0.50f	2.20±0.1cd	2.53±0.55ef	190.65±53.06d	193.75±40.74bc	70.
P value		0.0011	0.0001	0.0300	0.0005	0.0066	0.0110	0.0009	0.0

TABLE 7 Effect of biogenic NPs priming on seed germination parameters of flax on 7th day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Nanoparticle	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)
Non(Control)	0	93.33±1.87a	3.91±0.20d	2.75±0.68h	6.66±0.75h	0.70±0.16f	622.20±70.25g	100.00±0.00c
C-ZnO NPs	50	86.61±2.42b	4.37±0.62c	4.50±0.57e	8.87±1.10ef	1.03±0.11c	769.10±96.07e	112.17±16.13bc
	100	86.66±2.52b	4.42±0.29c	5.47±0.75d	9.80±0.62de	1.22±0.23b	849.26±54.34d	115.46±7.65bc
	150	96.66±1.91a	4.44±0.37c	6.00±0.11cd	10.34±0.65d	1.33±0.12b	999.46±63.23b	114.84±9.69bc
NC-ZnO NPs	50	80.23±2.86bc	5.37±0.25a	7.50±1.58b	12.87±1.37b	1.40±0.36b	1032.96±110.46ab	137.82±6.41b
	100	80.00±2.45bc	4.62±0.47bc	6.00±0.40cd	10.62±0.75d	1.32±0.13b	850.00±60.00d	118.58±12.27bc
	150	83.33±3.33bc	4.12±0.25cd	4.575±0.65e	8.70±0.62f	1.11±0.18bc	724.97±52.26ef	105.76±6.41c
Zn NPs	50	86.66±2.12b	5.00±0.22ab	8.25±0.86a	13.25±1.25a	1.64±0.06a	1148.24±109.04a	128.20±10.46ab
	100	73.35±3.15d	5.00±0.00ab	6.37±1.25c	11.37±1.10c	1.28±0.33b	834.12±81.29d	128.20±10.46ab
	150	73.33±3.35d	4.62±1.18bc	5.62±0.62d	10.22±0.64d	1.32±0.57b	751.63±47.33e	118.58±30.29bc
MgO	50	76.66±4.26	4.79±0.50b	4.62±0.62e	9.37±0.75e	0.98±0.17c	718.68±57.49ef	121.79±12.82b
	100	80.30±2.86bc	4.37±0.47bc	3.87±1.10fg	8.45±1.50f	0.87±0.17e	662.47±120.45fg	114.17±12.27bc
	150	73.33±3.25d	5.00±0.40ab	4.12±0.75ef	9.12±0.75e	0.83±0.19e	669.13±54.99f	128.20±10.46b
Ag	50	90.05±1.98ab	5.37±0.62a	4.87±0.25e	10.25±0.50d	0.91±0.14d	923.01±45.02c	137.39±16.13a
	100	83.33±3.33bc	5.22±0.63a	4.75±1.32e	9.97±1.51d	0.91±0.26d	831.21±126.62d	133.97±16.40a
	150	81.00±2.33bc	4.85±1.25b	4.37±0.75e	9.12±1.84e	0.96±0.25cd	739.12±149.26e	121.79±32.26bc
Cu	50	90.00±1.93ab	4.62±0.47bc	5.87±0.62c	10.50±0.91d	1.27±0.16b	945.00±82.15bc	118.58±12.27bc
	100	76.26±4.25d	3.87±0.47d	2.65±0.47h	6.50±0.40h	0.69±0.17f	498.29±31.29i	99.35±12.27cd
	150	76.66±4.25d	4.25±0.28c	2.77±0.85h	7.12±0.62g	0.68±0.24f	546.20±48.23h	108.97±7.40c
Fe	50	86.56±2.52b	3.37±0.47e	2.37±0.62i	5.75±1.04i	0.69±0.14f	498.29±90.19i	86.53±12.27e
	100	76.36±4.26d	4.12±0.62cd	2.75±0.61h	6.87±0.75gh	0.68±0.21f	537.03±57.49h	105.76±16.13c
	150	86.64±2.42b	4.12±0.47cd	2.37±0.77i	6.50±0.00h	0.59±0.19g	553.29±0.00h	105.76±12.27c
P value		0.0000	0.0100	0.0007	0.0001	0.0033	0.0082	0.0004

TABLE 8 Effect of metal salt (precursors) priming on seed germination parameters of flax on 2nd day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Metal salt	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RLSI (%)
Non(Control)	0	86.66±2.52bc	0.32±0.04d	2.24±0.28b	2.56±0.2cd	7.13±1.31a	221±24bc	100.00±0.00c	100.00±0.
$Zn(CH_3CO_2)_2$	50	96.66±1.91a	0.52±0.10a	2.62±0.35ab	3.10±0.3b	5.28±1.52c	303±31a	164.50±34.23a	116.96±1
	100	93.03±1.87ab	0.48±0.08b	2.56±0.28ab	3.04±0.1b	5.40±0.81c	283±30ab	147.00±26.14b	114.28±1
	150	91.00±1.93ab	0.42±0.08bc	2.12±0.88b	2.54±0.7cd	5.27±2.15c	228±79bc	131.25±26.4bc	94.64±39.
MgSO4	50	83.33±3.33bc	0.52±0.04a	2.52±0.25ab	3.04±0.2b	4.89±0.78d	255±19b	164.50±13.97a	112.50±1
	100	83.33±2.22bc	0.40±0.12bc	2.80±0.57a	3.2±0.9ab	7.25±1.49a	266±53b	125.00±38.2bc	125.00±2
	150	86.66±2.11bc	0.54±0.16a	2.72±0.31ab	3.26±0.35a	5.58±2.28bc	282±30ab	168.75±52.29a	121.42±1
AgNO3	50	86.66±2.52bc	0.54±0.15a	2.88±0.61a	3.42±0.54a	5.98±2.96b	297±46a	167.75±47.39a	128.57±2
	100	66.66±2.54d	0.26±0.31e	0.56±0.32ef	0.82±0.6fg	2.00±0.88f	54±41f	81.25±97.82d	25.00±14.
	150	0e	0f	0g	0h	0g	0g	0f	0h
CuSO4	50	86.66±2.52bc	0.40±0.07bc	1.30±0.29d	1.70±0.29e	3.34±0.92e	147±25d	125.00±22.9bc	58.03±13.
	100	93.66±2.71ab	0.22±0.16e	0.70±0.12e	0.92±0.2f	3.27±1.88e	86±24e	68.75±51.34e	31.25±5.4
	150	90.33±3.25b	0.32±0.08d	0.58±0.16ef	0.90±0.1f	1.97±0.76f	82±14e	100.00±26.14c	25.89±7.3
FeCl3	50	89.66±1.77b	0.46±0.08b	2.36±0.35b	2.82±0.29c	5.46±2.21c	255±26b	143.75±27.95b	105.35±1
	100	91.33±3.25ab	0.38±0.08c	1.82±0.20c	2.20±0.5d	5.03±1.41cd	198±14c	120.75±6.14bc	81.25±9.1
	150	87.00±2.12bc	0.46±0.13b	2.58±0.29ab	3.04±0.4b	5.86±1.12b	264±36b	143.85±41.92b	115.17±1
P value		0.0111	0.0001	0.0008	0.0039	0.0007	0.0004	0.0100	0.0006

TABLE 9 Effect of metal salt (precursors) priming on seed germination parameters of flax on 7[th] day, under different nanoparticle concentrations. Data are presented as mean values ±SD for three independent experiments. The same letters within a column show no significant difference at a 95% probability level at the $p < 0.05$ level.

Metal salt	Concentration (ppm)	Germination percentage (G%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	SVI	SLSI (%)	RLSI
Non(Control)	0	93.33±1.87a	3.91±0.20de	2.75±0.68h	6.66±0.75g	0.70±0.16e	622±70f	100.00±0.00d	100.0
$Zn(CH_3CO_2)_2$	50	86.66±2.52b	5.87±0.7a-c	7.12±1.43a	13.00±1.77a	1.20±0.19bc	112±154i	150.64±12.27b	259.0
	100	86.66±2.52b	4.25±0.50d	5.50±0.70cd	9.75±0.64d	1.35±0.26b	844±55de	108.97±12.82d	200.5
	150	80.00±2.45bc	4.25±0.86d	5.87±1.10c	10.12±1.43cd	1.43±0.38b	810±114de	108.97±22.20d	213.6
MgSO4	50	83.33±3.33bc	5.00±0.31c	5.81±1.21c	10.81±1.06c	1.17±0.31c	901±88c	128.20±10.46c	211.3
	100	83.33±3.33bc	6.75±3.27a	6.37±2.46b	13.12±3.77a	1.11±0.65c	1093±314a	173.07±84.06a	231.8
	150	90.00±1.93a	5.12±1.25c	5.75±0.50c	10.87±0.94c	1.20±0.39bc	978±85bc	131.41±32.05bc	209.0
AgNO3	50	86.66±2.52b	5.62±0.47bc	6.25±0.64b	11.87±0.62b	1.12±0.19c	1029±54b	144.23±12.27b	227.2
	100	40.00±4.65e	0.97±0.75g	1.92±1.86i	2.90±2.57i	1.67±1.03a	116±103i	25.00±19.45g	70.00
	150	0f	0h	0k	0j	0h	0j	0h	0i
CuSO4	50	76.66±4.26c	5.00±0.40c	3.50±0.70g	8.50±0.70e	0.70±0.17e	651±54f	128.20±10.46c	127.2
	100	73.33±3.25cd	3.37±0.85e	4.62±0.28e	4.00±0.62h	0.24±0.14g	302±46h	86.53±21.89e	27.26
	150	80.03±2.86bc	3.12±0.25ef	0.75±0.28j	3.97±0.47h	0.23±0.08g	310±38h	80.12±6.41ef	27.27
FeCl3	50	81.00±2.33bc	5.75±0.50bc	5.00±0.70d	10.75±1.19c	0.86±0.05d	870±96d	147.43±12.82b	181.8
	100	70.66±1.33cd	5.25±0.50c	2.87±1.10h	8.10±0.47ef	0.58±0.30ef	574±33g	134.61±22.20bc	104.5
	150	80.30±2.86bc	6.00±0.57ab	4.25±0.86ef	10.25±0.86cd	0.71±0.17e	823±69de	153.84±14.80b	154.5
P value		0.0044	0.0400	0.0006	0.0007	0.0055	0.0050	0.0001	0.000

Discussion

Effect of biogenic NPs and their counterpart salts on physiological characteristics of wheat seedling

As seed germination is the first step to start a successful crop improvement, it could be considered as an index to assay the enhancive or inhibitive effect of newly developed agrochemicals such as nanomaterials (Ahmed et al., 2019).

In present study, the seeds were exposed to the NPs or salts only for 12 hours but the priming effect was observed up to several days. In this regard, it could be suggested that the NPs or metal ions are absorbed on the surface of the seeds and gradually release to show their effect during a period of seven days. Also, the reason of difference between response of nano-primed seeds with salt-primed ones may be illustrated as the result of gradually release of ions from NPs by sub-toxic levels rather than the exposure to a large number of ions in the case of priming with metal salts which may cause stress in the germination process (Szollosi et al. 2020).

Previous findings have reported that the zinc nanoparticulate priming were more effective than zinc salt in enhancing the seedling growth. For instance, it is found that Zn NP treated wheat seeds surpassed elemental Zn values over $ZnSO_4$, indicating that NPs are more efficient at delivering Zn to plant tissues than $ZnSO_4$, which suggests it is done during a particle-specific mechanism (Baddar et al. 2018). Similar studies have shown that accumulation of Zn from NP treatment was more than predicted values upon dissolved Zn concentration (Ahmed et al. 2019). However, Zn is an essential metal for plant growth, it may be a phytotoxic metal when exceeds the tolerance limit depending on the plant species or plant's studied part (Zaeem et al. 2020).

Previous studies also reported similar results to our findings, on seedling growth of ZnO treated wheat seeds (Rawat et al. 2018; Awasthi et al. 2017). In the study conducted by Ahmed et al., C-ZnO NPs with very high concentrations of 0.05, 0.5, 2, 5 mg/ml were applied on four different seeds such as radish, cucumber, tomato and alfalfa to study the toxicity effect of NPs on seeds. They reported that C-ZnO exhibited no obvious toxic effect on germination, root and shoot growth of these seeds (Ahmed et al. 2019). Similarly, in our study C-ZnO and NC-ZnO NP treatment improved the seedling growth. Several mechanisms could be found in the literature illustrating various effects of NPs on plant parts and cell reactions. For example, the effect of NPs on specific enzymatic reactions and different enzymes such as amylase could elucidate NPs effect on seed germination. It is not clear at this point whether NPs toxicity is stimulated by particles or the dissolved ions (Ko et al. 2017). The effect of NPs may also be due to the interaction of NPs with some parts of the plants such as cell wall or membrane components. The size of the NPs is consistent with structure of the plant cell wall to enter the cell, at the point that the accumulation of reactive oxygen species (ROS) can be started (Zaeem et al., 2020). ROS can influence the permeability of the cells as it is interfered with the plasma membrane. Consequently, more NPs can result in intense stress after reaching the cells and stimulating the formation of stress-induced secondary metabolites (Zaeem et al. 2020).

In another study, Ag NPs toxicity on rockcress seeds was shown to be dependent on the size and concentration (Szollosi et al. 2020). Ag NPs with size of 80nm were only deteriorative at higher concentrations and those of 20 and 40 nm resulted in severe root growth inhibition. The researchers supposed that Ag NPs apoplastically transported through the root tissues (Szollosi et al. 2020). The inhibitory effect of Ag NPs on the germination index was also seen in the case of cucumber (Szollosi et al. 2020). Similar results are reported by Vanninia et al. (2014) as 10 ppm concentration of Ag NPs influenced the seedling growth of wheat seeds adversely. They also reported induction of morphological modifications in root tip cells by Ag NPs. According to the microscopy of the treated seeds roots, Ag NPs did not enter the root cells and located in the outer cells of the root cup. It was suggested by TEM analysis, that the toxicity effect of Ag NPs is resulted from release of Ag^+ ions from Ag NPs (Vanninia et al., 2014). Abbasi Khalaki et al. (2016) reported an enhancement in seedling growth of *thymus kotschyanus* seeds treated with 20 and 60% concentrations of Ag NPs.

The results reported by Zakharova et al. (2019) is comparable with our obtained results, in which wheat seeds were soaked in the presence of CuO NPs. They also reported that exposure of wheat seeds to 10ppm CuO NPs showed a 14.5% improvement in germination and a twofold increase in root and shoot length in comparison with control. At higher concentrations of CuO NPs, both stimulation and toxic effects were observed (decline in root length) (Zakharova et al. 2019).

The effect of wheat seed treatment with Cu NPs on germination and seedling vigor index has been studied by Yasmeen et al. under laboratory conditions (Yasmeen et al. 2015). Germination percentage, root and shoot lengths were calculated and the results indicated exposure of wheat seeds to Cu NPs lead to a decline in germination percentage and severe reduction of root and shoot length. Therefore, Cu NPs adversely affect germination and growth of wheat seeds (Yasmeen et al. 2015). This substantial decrease in the plantlet growth is consistent with previous wheat field studies, where the application of excessive NPs resulted in reduced plantlet length and distorted plantlet physiology (Du et al. 2011).

Plaksenkova et al. (2019) studied the effect of Fe_3O_4 NPs stress on the growth and development of rocket seeds. According to the results, 1ppm, 2ppm, and 4ppm concentrations of Fe_3O_4 NPs have positive effect on the growth and development of rocket seedlings. In a similar research, Yi Hao et al. studied the effects nano-priming with different Fe_2O_3 morphologies like Fe_2O_3 nanocubes, Fe_2O_3 short nanorods, Fe_2O_3 long nanorods at the concentrations of 5 to 150ppm on rice seeds during the germination. They found that all NPs considerably stimulated the root growth, and promoted shoot length at most concentrations while Fe_2O_3 long nanorods inhibited the seeds germination significantly and showed different biological effect from other Fe_2O_3 nanomaterials, due to their different shape (Hao et al. 2016).

Ngo et al. (2014) studied the effects of Fe NPs on soybean germination, growth, crop yield and product quality. In laboratory conditions, the germination rates of soybean seeds soaked with Fe NPs was 80% at the concentration of 0.08g/ha, while germination of the control sample was 55%. Consequently, various data obtained from such experiments could be really helpful in developing priming treatments for field experiments and other agricultural purposes.

Effect of biogenic NPs and their counterpart salts on physiological characteristics of flax seedling

The special structure of flax seed's coat was the reason we chose this plant for our experiments. The envelope or testa of the flax seed contain about 15% of mucilage, that mainly contains distinct types of arabinoxylans and water-soluble hydrocolloid/polysaccharides, which contribute to its gel qualities by forming large aggregates in solution (Mehtre et al. 2017). It is suggested that nano-priming of the flax seeds and also the NPs behavior may be affected by mucilage of the seed coat due to the thick chemical environment in which NPs were trapped. Therefore, according to the obtained results, it could be considered as one the reasons that we observed different results of flax seeds seedling in comparison with wheat seeds.

There are a few works studying the influence of NPs on seedling parameters of flax seeds. In support of our results, the effect of different metal and metal oxide NPs have been presented. As an example, it was reported that the application of biosynthesized MgO NPs enhanced the seed germination and growth parameters of peanut seeds as compared with control. The authors by using physicochemical methods including UV and SEM analyses, indicated that the MgO NPs penetrates into the seed coat, support water uptake inside the seeds, and then affect seed germination and growth rate mechanism (Jhansi et al. 2017).

In contrast of the results obtained from our research, Gorczyca et al. noticed that the 100ppm of Ag NP treatments applied to flax seeds had a limited effect on the germination and early development of the seedlings in comparison with the control. The response of the flax seeds to the NPs was reported as an increase of chlorophyll content (Gorczyca et al. 2018). Zaeem et al. (2020) investigated the effect of green synthesized C-ZnO NPs at concentrations of 0, 1, 10, 100, 500 and 1000ppm on growth of flax seeds. All the treated flax seeds had root development of different lengths, ranging from 2.62cm (for 1000 ppm ZnO NPs) to 7.08cm (for 10ppm ZnO NPs) with 3.85cm for the control. These results indicate efficiency of different concentrations of ZnO NPs in seed germination. At ZnO NPs concentration of above 10ppm, the higher the concentration of NPs, the lower the root length. The increased sensitivity of radicle to NPs is due to the large surface area of the NPs. They suggested that the observed inhibitory effect on seed germination may be because of the very small size of NPs and the dissolution power of ZnO to Zn^{2+} ions (Zaeem et al. 2020).

To the best of our knowledge, there was not any report on positive or negative effect of Cu NPs on flax seedling to be compare with our findings. In previously reported researches, despite our findings, stimulating effects of Fe NPs on seedling of different species have been described, for example in rocket (Ko et al. 2017), rice (Hao et al. 2016) and soybean (Ngo et al. 2014). Clearly, different results obtained from flax seed priming in comparison to the wheat seeds in germination and seedling growth parameters.

A comparison between the effect of biogenic NPs and their counterpart salts on physiological characteristics of wheat and flax seedlings

At 7th day of the experiment, in a comparison between the effect of studied NPs in applied concentrations, the most effective one in shoot and root development was Zn NP and the less effective NPs were Ag for wheat and Fe for flax seeds (Fig. 2). It shows Zn NPs are not toxic at the applied concentrations and even show stimulating effect on both wheat and flax seeds. Vice versa the applied concentrations of Ag NPs are toxic for wheat but stimulating for flax. As all of the experiment conditions are the same for all of the samples, it could be concluded these differences are related to the seed species. Among the tested metal slats, zinc acetate had the most stimulating effect and $CuSO_4$ was the most toxic one for both flax, and wheat. Nano forms of metals and metal oxides, have been reported to significantly improve root or shoot elongation and seed germination of wheat in comparison with bulk materials (Feizi et al. 2012). This kind of growth development mainly depends on the concentration of NP, duration of nano-priming, growth medium and species of plant (Ahmed et al. 2019).

Among measured parameters, root length is more sensitive than shoot length. Between wheat and flax roots, the flax root length was more sensitive against NP and salt treatments and wheat shoot length was the less sensitive parameter. All over, flax seeds were more sensitive to the treatments compared to the wheat seeds. Although the factors which impact the root and shoot elongation following NP exposure are not clear yet, it could be suggested that the polymeric network of flax seeds mucilage trap NPs or metal ions and then the accessibility of them for flax seeds differs from wheat seeds in the period of our experiment (Ahmed et al. 2019).

Figure 3 provides an image for better comparison between germination percentage variations with changes in concentrations of used NPs and salts. In majority of the cases wheat seeds had more G% than flax seeds. 150ppmof $AgNO_3$ solution had such a toxic effect on flax seeds that no germination was observed in this treatment. Similarly, metal-based NPs have been reported to show dual impacts on plants growth, such as seed germination. Positive effects of metal-based NPs treatments were displayed in different plants (Szollosi et al. 2020). Seed germination of soybean seeds enhanced by nano-priming with Co, Fe and Cu NPs (Mehtre et al. 2017), also similar findings were reported in the case of some Solanaceae crops after treatment with ZnO and TiO_2 NPs (Younes et al. 2020). The obtained results were comparable with those reported by Feizi et al. (Feizi et al., 2012), in which seed treatment with TiO_2 NPs at low concentrations (1-2ppm) resulted in an improve in germination of wheat seeds and also seedling elongation compared to untreated wheat, but no significant effect at concentration of 100ppm was observed (Feizi et al., 2012).

Conclusion

Suggesting that using green synthesized nano-size minerals for seed treatment could be helpful for seedling growth improvement. Present study provides new information on possible positive or toxic effects of seed priming with NPs on germination percentages, shoot length, root length, seedlings length, root/shoot ratio, seedling vigor index (SVI), shoot length stress tolerance index (SLSI) and root length stress tolerance index (RLSI) which were calculated at 2nd and 7th days on two popular early growth plants, wheat and flax. Plant's dual responses varied among NPs type and correlated to the tested concentrations. According to the obtained results, the response of the tested plants to a certain NP was different between flax and wheat. Moreover, it differed between applied concentrations of the NPs. For example, Ag NPs showed a significant positive effect on root and shoot elongation of flax seedlings but there were a dose dependent decrease of root and shoot elongation of wheat seedlings over their respective controls. Another important result is that flax seed was more sensitive to priming with metal salts and NPs in comparison with wheat seed. Furthermore, the influence of these treatments was investigated in earlier stages of the growth, in 2nd day of the experiment, in comparison with 7th day of the experiment. Among the studied NPs, Zn and Ag NPs exhibited the best biological effects on growth and development of wheat and flax respectively. The effect of the nanoparticle's counterpart metal salts on seedling parameters also studied for comparison with nanoparticulate ones. Over all, nanoparticle treatments were more effective than metal salt treatments in root and shoot development. The basic mechanisms need to be investigated in future investigations.

Declarations

Author contribution

M. B. conducted the majority of the experiments, performed results interpretation as well as manuscript writing, performed experiments; M.Z. performed statistical analysis, contributed to results interpretation and reviewed the manuscript, contributed to the overall design of the experiments, results interpretation, manuscript writing, and reviewed the manuscript. Both authors read and approved the final manuscript.

Supplementary Information

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Availability of data and materials

The datasets used and/or analyzed 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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Figures

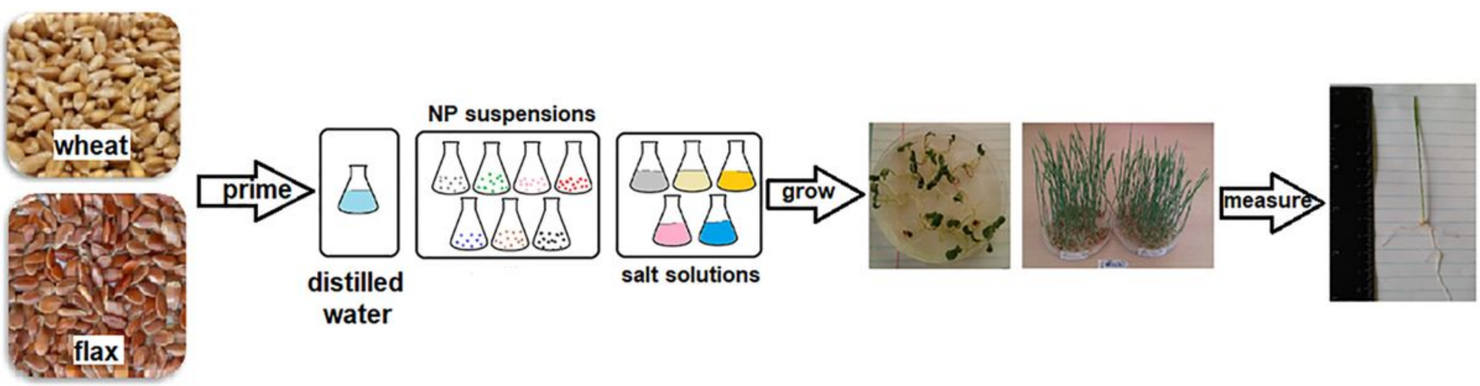

Figure 1

Graphical summary of the experiment

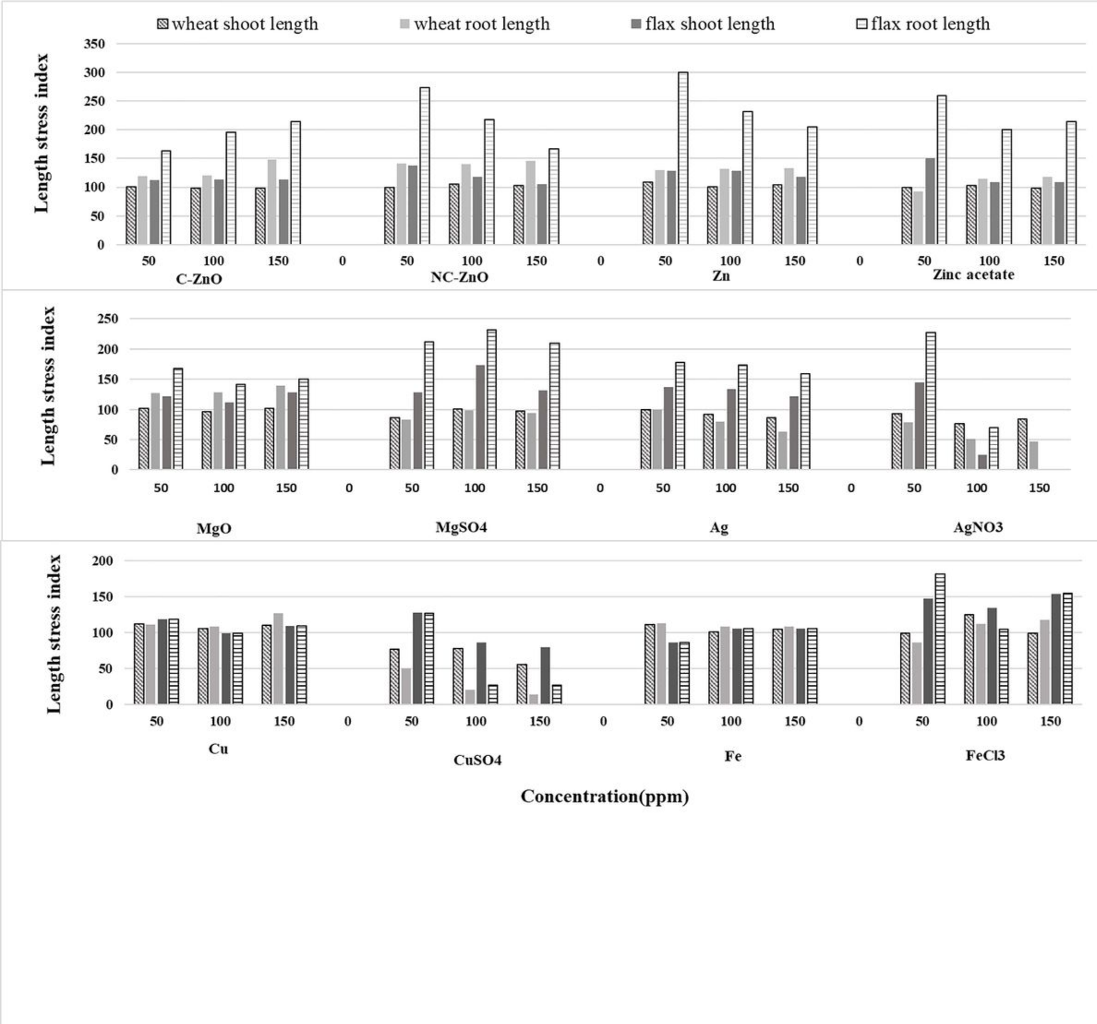

Figure 2

Dose response effect of NPs and their correspondent metal salts on shoot and root stress tolerance index (SLSI and RLSI, respectively) of wheat and flax seeds at 7th day of the experiment.

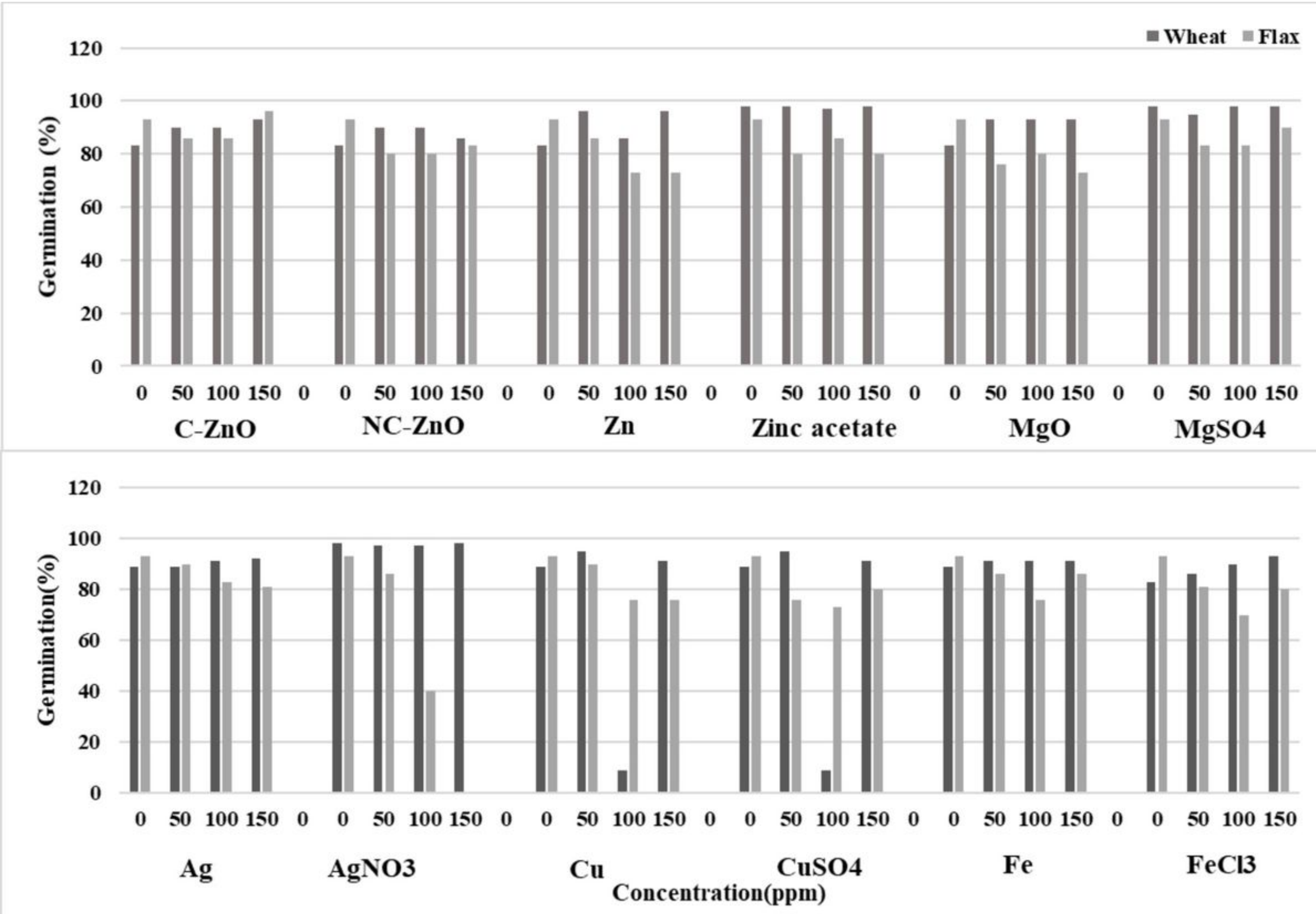

Figure 3

Dose response effect of NPs and their correspondent metal salts on germination percentage of wheat and flax seeds at 7th day of the experiment.