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Screening of the effects of Zinc oxide based nanofertilizers on the germination of *Lathyrus sativa* L. seeds

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Summary

Zinc based nanofertilizers may be useful tools in improving crop culture, especially in Zinc deficient soil. The present study aims to investigate the role of nanosized zinc oxide particles (ZnO NPs, diameter<100 nm) in modulating seed germination, embryo nutrition and growth of grass pea (*Lathyrus sativus* L.). Our data revealed ameliorating or inhibiting effects depending of the concentration of ZnO NPs administrated. At metabolic level, the growing embryonic axes seem to cope with induced oxidative stress, by enhancing hydrogen peroxide scavenging capacity. We revealed interesting regulatory mechanisms evolved within the embryonic cells to limit the oxidative damages induced by ZnO NPs and Zinc sulfate when applied at low concentrations (0.01 mg mL^{-1}, 0.1 mg mL^{-1}). Nonetheless, at high concentrations (1 mg mL^{-1}, 10 mg mL^{-1}), ZnO NPs led to drastic perturbations in the metabolism, which resulted in the inhibition of root and seedling growth. Our work may bring novel insight into the mechanistic understanding of the physiological role of the nanosized ZnO in enhancing the efficacy of fertilization. We also assess the critical role of applied concentration of nanofertilisers to avoid toxicity to plants. Such phytotoxicity is not only affecting crops yield, but also may alter the biological properties and the nutritive quality of plant-derived food products, which may endanger or risk human health.

Keywords: Germination, *Lathyrus sativus* L., Nutrition, Response, Zinc oxide nanoparticles.

Introduction

Zinc oxide nanoparticules (ZnO NPs) are nanoscale (1-100 nm) materials, with novel applications in cosmetics, bioimaging, drug delivery, health care, bioremediation, environmental and bioprocess control (Naveed et al., 2017; Keskinbora and Jameel, 2018). In agriculture, nanotechnology provides novel "tools" to improve plant nutrient, productivity and resistance against the environmental stresses (Mahakham et al., 2017). ZnO NPs were used as nanopesticides and nanofertilizers (Kah, 2015). Nanofertilizers have the capacity to synchronize the release of nutrients with their plant uptake, which can decrease nutrient loss and limit the risk of groundwater contamination. They can offer efficient delivery systems to plants via encapsulating nutrients, and their selective discharge to be directly internalized by the plant (Prasad et al., 2010). This also limits nitrogen and nutrients loss due to leaching, emissions, and long-term assimilation by soil microorganisms (Tarafdar et al., 2014).

On the other hand, Zinc (Zn) is one of the essential micronutrients required for proper growth and yield of crops in plants. It is required for germination, and it is involved in maintaining the structural and functional integrity of cells and proteins (Das et al., 2016). Zn also serves as a cofactor for many structural and catalytic proteins (Romeo et al., 2014). Hence, Zn deficiencies can lead to negative effects on all actors of the whole chain, notably humans. This results in the impetus on the importance to improve the uptake of Zn by crops and subsequently humans. Therefore, ZnO NPs were shown to be efficient in improving plants growth (Rizwan et al., 2018), as well as in promoting plants defense against environmental abiotic and biotic stresses (Kah, 2015). Nonetheless, the use of agrochemicals and fertilizers may cause the accumulation of Zn ions in the agricultural soil (Kah, 2015). Besides, the mechanisms by which NPs exert their effects in plants are not well clear, and heavy metals released from metal- and metal oxide NPs can be a major point of concern (Owen and Handy, 2007). In literature, differential effects of NPs on the plant biological pathways were shown to be dependent on their physical and chemical properties (surface coating, type, size), their concentration, bioavailability, methods of exposure, as well as the plant species and life cycle stage (Raliya et al., 2015; Dimkpa et al., 2020). For instance, the phytotoxicity of ZnO NPs to plants has been reported in many studies (Lee et al., 2010; Dimkpa et al., 2020). Indeed, among the negative effects of metal oxide NPs are the inhibition of crops yield and biomass (Ebbs et al., 2016; Bradfield et al., 2017). They also induce cellular and genetic toxicity, via the generation of ROS. The phytotoxicity of ZnO NPs has been shown on many plant species, such *Triticum aestivum* L. (Yahyaoui et al., 2017) and *Pisum sativum* L. (Mukherjee et al., 2014).

When ZnO NPs are dissolved in water, they release Zn^{2+} ions, which are one of the principle sources of toxicity. This toxicity can be added to a supplementary toxicity due to nanosized particles themselves. Excess of Zn ions also resulted in toxicity, via the alteration of proteins function and activity, and the disruption of cellular metabolism, expression of genes involved in metal homeostasis and binding, thus leading to cell death (Mustafa and Komatsu, 2016).

The current study aims to investigate the effects of ZnO NPs on the germination of grass pea (*Lathyrus sativa* L.) seeds, particularly the growing embryonic axes. *Lathyrus sativus* L. is a legume species of Fabaceae, cultured as human food and animal feed, for its important nutritional and economic properties, mostly in the rgions of India, Asia, South America, Mediterranean, South Europe and North of Africa (Hanbury et al., 2000). Grass pea seeds are characterized by 48% of starch, 1% of lipids and 28% of proteins (Xu et al., 2017), including low contents in cystein and methionin, and high content in lysine and threonine (Yan et al., 2006). Nonetheless, *Lathyrus* contains high level of neurotoxinem known as β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP), which is responsible of lathyrism (Grela et al., 2001). Jiao et al. (2006) also showed that Zn deficiency caused the increase of β-ODAP. *Lathyrus sativus* L. is, however, able to thrive in arid and semi-arid areas, in several tropical

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and subtropical countries, therefore it is well adapted to unfavorable agricultural conditions, such as floods, drought, salinity, low soil fertility, infected soils and pathogens (HANBURY et al., 2000).

In the present study, the response of *Lathyrus sativa* L. germinating seeds to increasing concentrations of ZnO NPs, commonly used as nanofertilizers, was evaluated. The eventual mechanisms by which ZnO NPs may interfere at the physiological and metabolic levels were discussed by the comparison between the assays of nanosized ZnO NPs and soluble Zn ions (treatment with Zn sulfate, $ZnSO_4$).

Materials and methods

Treatments, germination and embryo growth

Seeds of *Lathyrus sativa* L. were surface sterilized by sodium hypochlorite 20% for 2 min, followed by ethanol 70% for 2 min, and thouroughtly washed with distilled water. Seeds were then germinated on filter paper in the presence of distilled water (control), or solutions of Zinc oxide nanoparticles (ZnO NPs) and zinc sulfate ($ZnSO_4$), at concentrations of 0.01 mg mL^{-1}, 0.1 mg mL^{-1}, 1 mg mL^{-1} and 10 mg mL^{-1}. For each treatment, we carried 6 petri-dishes, with 10 seeds per petri-dish (six biological replicates/ treatment). Germination was performed in culture room in dark at 28 °C. The solutions of ZnO NPs were dispersed by vortexing and sonication, to avoid NPs aggregation or precipitation. Same volumes of 5-10 mL of distilled H_2O, and solutions of ZnO NPs and $ZnSO_4$ were added daily, in order to maintain seeds imbibition. The whole experiment was repeated as three technical replications.

After 4 days, the length of roots and embryonic axes were measured. Fresh biomass (FM) of embryos was measured. The embryonic axes were stored at -20 °C, to use for the biochemical assays. Other samples of embryonic axes were dried at 60 °C in Oven (Thermoline Scientific) to constant weights, then dry biomass (DM) was measured, and used for the determination of mineral content.

All the chemicals, reagents ($ZnSO_4$, ZnO NPs diameter <100 nm) were purchased from Sigma-Aldrich.

Measurement of levels of hydrogen peroxide (H_2O_2)

The embryonic axes were homogenized in the presence of 0.1% (w/v) trichloroacetic acid (TCA) (1:20, w/v), then the homogenate was centrifuged at 12 000 × g for 20 min at 4 °C. An aliquote of supernanant was added to 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (KI). Levels of H_2O_2 were determined by measuring the absorbance at λ = 390 nm (SERGIEV et al., 1997).

Assays of antioxidant enzymes

The embryonic axes were homogenized in the buffer containg 25 mM potassium phosphate (KH_2PO_4/K_2HPO_4), pH 7.0 and 5 mM sodium ascorbate. Homogenate was centrifuged at 20 000 × g for 30 min, using the Sorvall X1R general purpose refrigerated centrifuge (Analytical bioNanoTechnology Equipment Core (ANTEC), according to manufacture instructions. The resulting supernatant was considered as the enzymatic extract.

Catalase (CAT; EC 1.11.1.6) activity was measured by monitoring the decrease of absorbance at 240 nm ($\varepsilon_{240} = 36 \times 10^{-6}\ M^{-1}\cdot cm^{-1}$) (AEBI, 1984). The reaction consisted in 25 mM KH_2PO_4/K_2HPO_4 (pH 7) and 10 mM H_2O_2.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured in the reactional mixture containing 50 mM KH_2PO_4/K_2HPO_4 (pH 7), 0.5 mM sodium ascorbate, 5 mM H_2O_2 and 0.1 mM EDTA. The decrease of absorbance at 290 nm was measured using the extinction coefficient $\varepsilon_{290} = 2.8 \times 10^{-3}\ M^{-1}\cdot cm^{-1}$ (NAKANO and ASADA, 1981).

Guaiacol peroxidase (GPOX; EC 1.11.1.7) activity was measured by monitoring the increase of absorbance at at 470 nm ($\varepsilon_{470} = 26.6\ M^{-1}\cdot cm^{-1}$) (FIELDING and HALL, 1987). The reactional mixture contains 10 mM H_2O_2 in 25 mM KH_2PO_4/K_2HPO_4 (pH 7.0) and 9 mM guaiacol.

Glutathione reductase GR (EC 1.6.4.2) activity was measured using 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0) buffer, 0.2 mM NADPH and 0.5 mM GSSG. The decrease of absorbance was measured at 340 nm ($\varepsilon_{340} = 6.22 \times 10^3\ M^{-1}\cdot cm^{-1}$, FOYER and HALLIWEL, 1976).

Activities were expressed in Units of enzyme activity: µmol min^{-1} mg^{-1} FM. All measurements were carried out using JENWAY 6405UV/Vis Spectrophotometer.

Determination of mineral elements by Atomic Absorption Spectrometry (AAS)

Dry samples of embryonic axes (0.1-0.2 g) were digested in a mixture of 4 M nitric acid (HNO_3) and 1 M perchloric acid ($HClO_4$), by heating process 500 °C. The resultant ashes were resolubilised in 0.7% HNO_3 to a final adjusted volume of 15 mL, and then filtered using cender free filter paper of 70 mm diameter. The obtained solutions were analyzed for the content of some mineral microelements (Zn, Cu, Mn, Fe) by AAS, according to manufacture instructions (Thermo SCIENTIFIC, Type 138 iCE3500AA System, NC942350023500, Ser No C103500104). The operation conditions used to operate AAS instrument were as recommended by the manufacturer. Data were rounded off properly based on the value of standard deviation from measurement conducted in triplicate. Final results are means of values obtained from three biological replicates.

Statistical analysis

Statistical analyses were carried out using SPSS 20.0 and *Xl*stat version 9.0 software 2014. Data were subjected to analysis of variance (two ways) ANOVA at α=0.05%, and Duncan post hoc multi-range test at 5%, to compare the effect of the treatments (ZnO NPs and $ZnSO_4$), the effect of concentrations, and the interaction of effects (treatment×concentration).

Results

Effects of ZnO NPs on the physiology of seed germination and embryo growth

In the present study, the effects of ZnO NPs on seeds of *Lathyrus sativa* L. were investigated. Our results showed variations of seed germination rate with the increasing concentrations of ZnO NPs (Fig. 1A) and $ZnSO_4$ (Fig. 1B). The variations between days were estimated significant ($p<0.05$) for each treatment separately (Fig. 1). In comparison with control, concentrations of 0.01 mg mL^{-1}, 0.1 mg mL^{-1} and 1 mg mL^{-1} enhanced germination at day 2, but concentration of 10 mg mL^{-1} decreased germination capacity. However, these differences between applied concentrations (0.01 mg mL^{-1}, 0.1 mg mL^{-1}, 1 mg mL^{-1} and 10 mg mL^{-1}) were estimated not significant. Also, differences between treatments (ZnO NPs and $ZnSO_4$) were found not significant. Similarly, at days 3 and 4, the delays in germination of 1 mg mL^{-1} and 10 mg mL^{-1} treated seeds were found not significant in comparison with controls. However, Fig. 2 showed that the length of embryonic axis (Fig. 2A) and the length of root (Fig. 2B) were enhanced significantly ($p<0.001$) at low concentrations (0.01 mg mL^{-1}, 0.1 mg mL^{-1}), whereas they were inhibited at higher ones (1 mg mL^{-1}, 10 mg mL^{-1}). The effect of treatments (ZnO NPs and $ZnSO_4$) was found not significant, but the effect of concentration was highly significant ($p<0.001$) (Fig. 2).

Furthermore, the negative effect of $ZnSO_4$ was found more pronounced than ZnO NPs, particularly when applied at high doses. Besides, the concentrations of 0.01 mg mL^{-1} and 0.1 mg mL^{-1} of both treatments resulted in embryonic FM increase. At higher concentra-

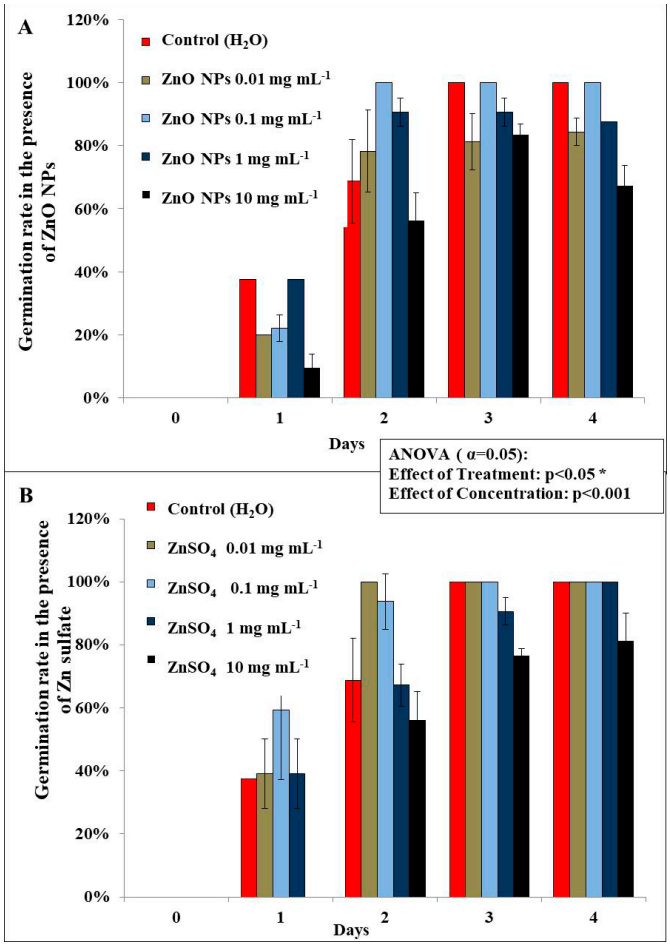


Fig. 1: Germination rate of *Lathyrus sativa* L. seeds during time (days) in the presence of distilled water (Control) or different concentrations of ZnO NPs and $ZnSO_4$; 0.01 mg ml^{-1}, 0.1 mg ml^{-1}, 1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) which are average of two indepandant germinations. Differences between treatments and within concentrations estimated according to Duncan test (α=0.05) were non significant (*P*<0.05).

tions of 1 mg mL^{-1} and 10 mg mL^{-1}, a highly significant (p<0.001) reduction of FM (Fig. 3A) was recorded. The effects of ZnO NPs on the DM of embryonic axes were not clearly noticeable, except a decrease at high concentrations (1 and 10 mg mL^{-1}). Variations of the cotyledonary FM were, however, detected in ZnO NPs treated seeds (Fig. 4A). These variations were estimated significant (p<0.01) with treatments and highly significant (p<0.001) with concentrations. No significant changes of the DM of cotyledons were registered with ZnO NPs or $ZnSO_4$ (Fig. 4B), which could be attributed to the short duration of assay; 4 days of germination might not be enough to detect or monitor the cotyledonary biomass changes, in the contrary of the embryonic axes.

Effects of ZnO NPs on the antioxidant status and mineral nutrition of the growing embryos

The effects of 0.1 and 10 mg mL^{-1} of ZnO NPs and $ZnSO_4$ were assayed on the antioxidative response of the growing embryonic axes. Fig. 5 revealed that the concentration 0.1 mg mL^{-1} of both treatments did not affect significantly the levels of H_2O_2 (hereby playing the role of assignaling molecules) in comparison with control. In the contrary, the higher concentration (10 mg mL^{-1}) caused a highly significant (p<0.001) increase in the level of H_2O_2 (herein becoming toxic molecules). Besides, Fig. 6 showed several changes within the activities of antioxidant enzymes; A reduction of CAT activity was estimated significant at p<0.01 as the effect of treatments ZnO NPs and $ZnSO_4$, and highly significant (p<0.001) as the effect of concentration (Fig. 6A). Combined effect of Treatment and Concentration was found significant at p<0.01. APX and GPOX activities (Fig. 6 B, C) showed highly significant variations (p<0.001) with the concentrations applied. APX activity (Fig. 6B) decreased in the presence of both treatments, particularly $ZnSO_4$. GPOX activity was however stimulated in the presence of 0.1 mg mL^{-1} ZnO NPs, and in the presence of $ZnSO_4$ (0.1 mg mL^{-1} and 10 mg mL^{-1}) (Fig. 6C). These variations were estimated highly significant (p<0.001) with Treatments, Concentrations and their combined effect. GR activity increased significantly with 10 mg mL^{-1} ZnO NPs and 0.1 mg mL^{-1} Zn SO_4 (Fig. 6D). Moreover, the evaluation of the levels of total soluble proteins revealed an increase in the presence of 0.1 mg mL^{-1} of ZnO NPs, but remained not significant, while at higher concentration of 1 mg mL^{-1} ZnO NPs a significant decrease was recorded (Fig. 7).

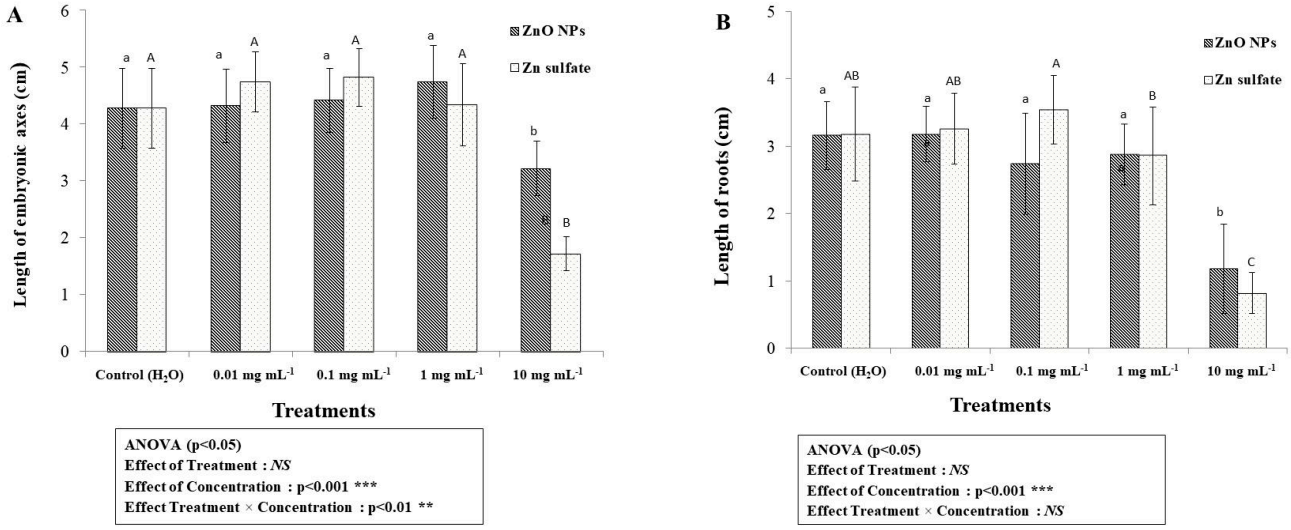


Fig. 2: Length of the embryonic axes **(A)** and the roots **(B)** of *Lathyrus sativa* L. seeds germinated for 4 days in the presence of distilled water (Control) or solutions of ZnO NPs and $ZnSO_4$ at concentrations 0.01 mg ml^{-1}, 0.1 mg ml^{-1}, 1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) of 5 independent measurements. Letters a,b and A-C denote statistic classes, respectively, for ZnO NP and Zn sulfate, using Duncan test (α=0.05). Differences are significative, at *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001, and non significant *NS* at P≥0.05.

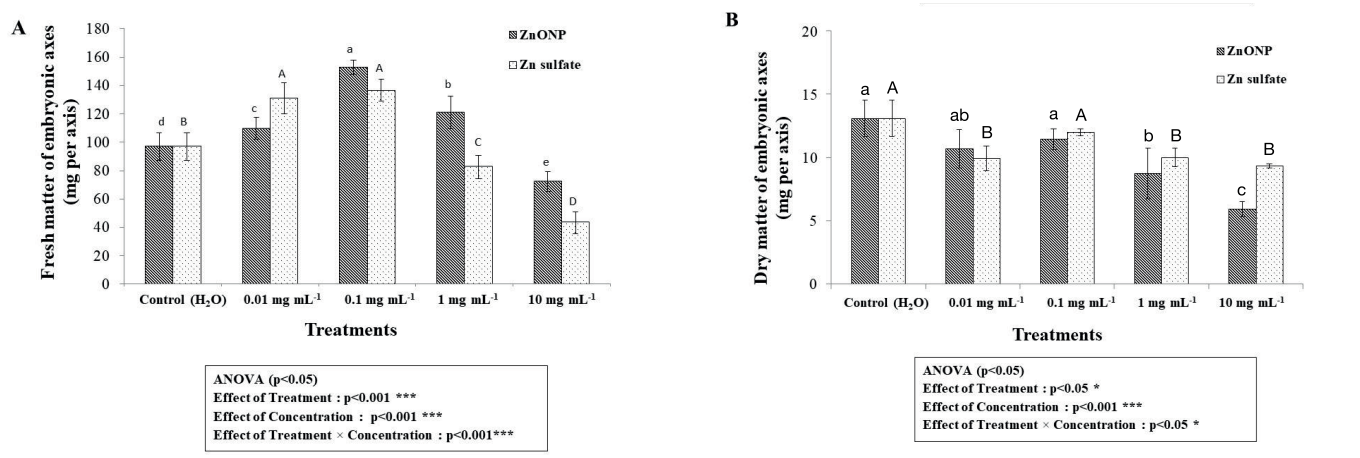


Fig. 3: Fresh matter **(A)** and dry matter **(B)** of the embryonic axes of *Lathyrus sativa* L. seeds germinated for 4 days in the presence of distilled water (Control) or solutions of ZnO NPs and $ZnSO_4$ at concentrations 0.01 mg ml^{-1}, 0.1 mg ml^{-1}, 1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) of 5 independent measurements. Letters a-c and A-C denote statistic classes, respectively, for ZnONP and Zn sulfate, using Duncan test (α=0.05). Differences are significative, at *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001, and non significant *NS* at P≥0.05.

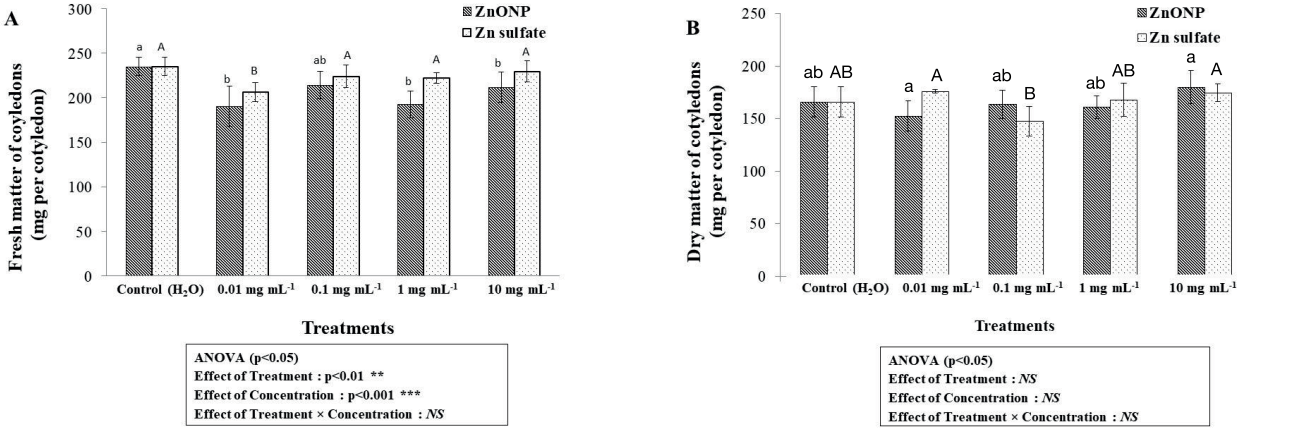


Fig. 4 Fresh matter **(A)** and dry matter **(B)** of the cotyledons of *Lathyrus sativa* L. seeds germinated for 4 days in the presence of distilled water (Control) or solutions of ZnO NPs and $ZnSO_4$ at concentrations 0.01 mg ml^{-1}, 0.1 mg ml^{-1}, 1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) of 5 independent measurements. Letters a-c and A-C denote statistic classes, respectively, for ZnO NPs and Zn sulfate, using Duncan test (α=0.05). Differences are significative, at *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001, and non significant *NS* at P≥0.05.

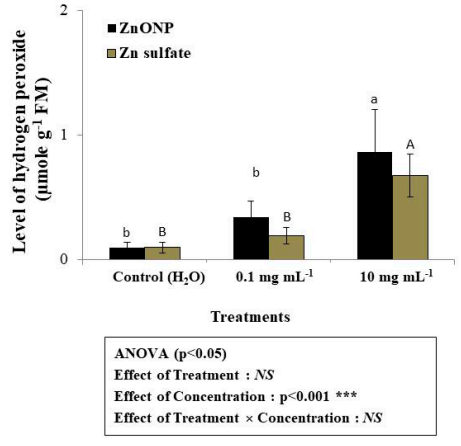


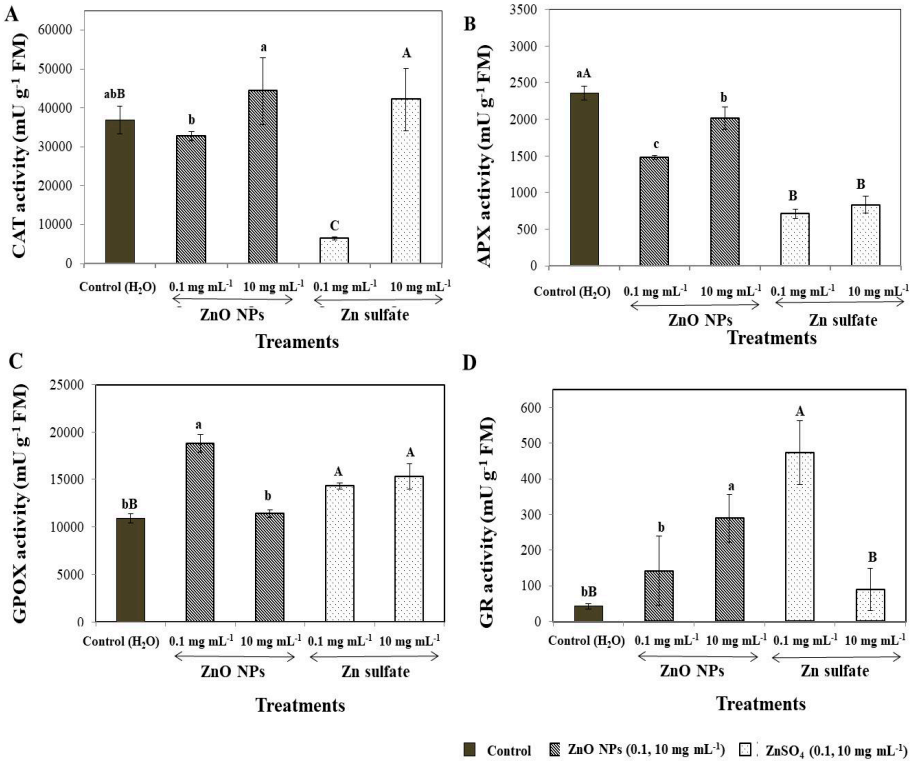
Fig. 5: Level of proxide hydrogen in the embryonic axes of *Lathyrus sativa* L. seeds germinated for 4 days in the presence of distilled water (Control) or solutions of ZnO NPs and $ZnSO_4$ of 0.1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) of 5 independent measurements. Letters a-b and A-B denote statistic classes, respectively, for ZnO NPs and Zn sulfate, using Duncan test (α=0.05). Differences are significative, at *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001, and non significant *NS* at P≥0.05.

In Zn SO_4 treated embryonic axes, differently to ZnO NPs, no significant variations were found with both concentrations of Zn SO_4 (Fig. 7).

Additionally, the determination of the content of Zn and other micronutrients (Mn, Fe and Cu) by AAS in the embryonic axes showed a significant increase in the accumulation of Zn ions particularly with the highest concentration of 10 mg mL^{-1} of ZnO NPs and $ZnSO_4$. Besides, a significant decrease in the content of Fe was found after exposure to ZnO NPs (Tab. 1). Similar decrease was also registered with the highest dose of $ZnSO_4$. Variation of Mn content was estimated not significant, except an increase with 0.1 mg mL^{-1} $ZnSO_4$. Similarly, Cu content decreased with 0.1 mg mL^{-1} and 10 mg mL^{-1} in both treatments, especially with 10 mg mL^{-1} $ZnSO_4$.

Discussion

The use of nanosized ZnO in fertilization represents a novel technique to improve the nutrition and growth of plants especially when cultured in the presence of critical soil and water factors. Nevertheless, the risk of a potential hazard of ZnO NPs on plant system is not none. In order to shed more light on this point, the effects of ZnO NPs on seeds of *Lathyrus sativa* L. were investigated. In order to ascertain wheither the effects of ZnO NPs can be attributed to Zn ions, seed



ANOVA results (p<0,05)	CAT	APX	GPOX	GR
Effet traitement (T)	**	NS	***	NS
Effet concentration (C)	***	***	***	***
Effet combiné T×C	**	***	***	***

Fig. 6: Activities of the antioxidant enzymes CAT **(A)**, APX **(B)**, GPOX **(C)** and GR **(D)** in the embryonic axes of *Lathyrus sativa* L. seeds germinated for 4 days in the presence of distilled water (Control) or solutions of ZnO NPs and $ZnSO_4$ at concentrations of 0.1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) of 5 independent measurements. Letters a-c and A-C denote statistic classes, respectively, for ZnO NP and Zn sulfate, using Duncan test (α=0.05). Differences are significative, at *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001, and non significant *NS* at P≥0.05.

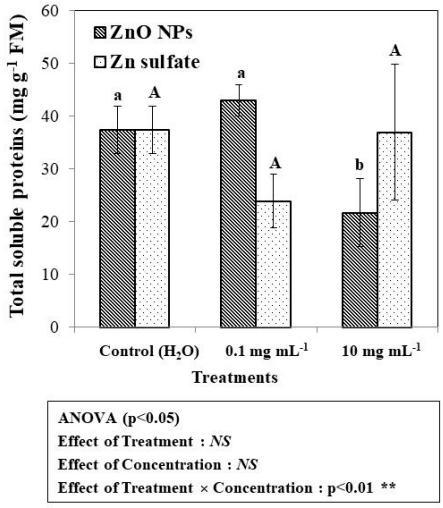


Fig. 7: Levels of total soluble proteins in the embryonic axes of *Lathyrus sativa* L. seeds germinated for 4 days in the presence of distilled water (Control) or solutions of ZnO NPs and $ZnSO_4$ at concentrations of 0.1 mg ml^{-1} and 10 mg ml^{-1}. Values are means (±SD) of 5 independent measurements. Letters a-c and A-C denote statistic classes, respectively, for ZnO NP and Zn sulfate, using Duncan test (α=0.05). Differences are significative, at *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001, and non significant *NS* at P≥0.05.

germinations in the presence of ZnO NPs and Zn sulfate were compared to scheme their mechanism of response. Besides, we aim to determine the concentrations of Zn nanofertilizers that are possibly improving growth or in contrary causing toxicity, which may bring insight at the choice of concentration to apply.

Our results revealed the significant interference of ZnO NPs and $ZnSO_4$ on the germinative process and the early growth of embryo, dependent on the applied concentration; indeed, low concentrations (0.01 mg mL^{-1}, 0.1 mg mL^{-1}) seem to promote seeds germination, as well as the elongation and biomass of the embryonic axis. Higher doses had, however, toxic and inhibitory effects (1 mg mL^{-1}, 10 mg mL^{-1}). Overall data showed that ZnO NPs affected negatively FM and DM of the embryonic axes, while Zn sulfate affected FM more than DM, which could probably be attributed to the process of water absorption following seed imbibition. We may hypothesize that the low doses of ZnO NPs and Zn sulfate induced the intense absorption of water, without significant modification of dry biomass, which can explain the increase of FM *versus* no change of DM, as compared with respective controls. Hence, this increasing absorption of water as compared with control could be an adaptative response in seeds to improve tolerance and defense mechanism against the metallic stress and the osmotic stress imposed by ZnO NPs or $ZnSO_4$. This leads to the hypothesis that seeds try to cope with stressfull condition, via the enhancement of a vital phenomenon associated with waper uptake, which may explain in part the finding that no significant variation was recorded for the length of embryonic axes and roots. This

Tab. 1: Content (mg L^{-1}) of mineral elements (Mn, Zn, Cu, Fe) in the embryonic axes of 4 day-germinated seeds, measured by atomic absorption spectrometry (AAS). Letters a-c and A-C denote statistic classes, respectively, for ZnO NPs and Zn sulfate, using Duncan test ($\alpha = 0.05$).

Treatment	Concentration	Mn	Zn	Cu	Fe
Control	-	1.263±0.079 aA	2.195±0.124 bB	1.3065±0.310 aA	3.536±0.025 aA
ZnONP	0.1 mg mL^{-1}	1.176±0.005 a	1.4735±0.044 c	1.1365±0.170a	1.1335±0.161b
	10 mg mL^{-1}	1.275±0.098 a	11.355±0.134 a	1.0555±0.054 a	1.731±0.463 b
Zn sulfate	0.1 mg mL^{-1}	1.397±0.031AB	1.642±0.043 C	0.9775±0.050 A	2.3355±0.159 B
	10 mg mL^{-1}	1.5065±0.021A	12.83±0.056 A	0.827±0.005 A	1.4915±0.086 C

ANOVA: all differences are significant at $P<0.05$.

adaptative response seems to be possibly disrupted or altered with the highest concentration (10 mg mL^{-1}) that triggers a significant decrease of the embryonic growth (Figs. 2, 3). Besides, the decrease of FM and DM at 10 mg mL^{-1} suggests that ZnO NPs may act via the inhibition of water absorption (similar effect with Zn sulfate), which brings evidence of the involvement of Zn^{2+} ions possibly released from ZnO NPs and Zn sulfate. Additionnally, embryo DM seems to be negatively affected more by ZnO NPs than $ZnSO_4$, which suggests that the reduction of embryo biomass could be the proper effect of NPs themselves. Consequently, NPs might interfere with the mobilization of biomass and allocation of nutrients in cotyledons.

In this study, overall data discussed above may suggest that *Lathyrus sativa* L. evidenced tolerance capacity towards ZnO NPs up to 10 mg mL^{-1}. Therefore, we may ascertain that lower concentrations of ZnO NPs are probably required as fertilizers. Our findings agree with other reports that the effects of Zn ions and ZnO NPs depend upon their concentration applied, and the biological properties of plant species, such as the permeability of seed coat to NPs and their internalization in root tissues (Dimkpa et al., 2020). Indeed, Upadhyaya et al. (2017) demonstrated that rice exposure to lower concentrations of Zn NPs (5, 10, 15, 20 and 50 mg L^{-1}) showed better potential of seed germination, as well as radicle and plumule growth. De la Rosa et al. (2013) also reported that ZnO NPs improved germination capacity of cucumber seeds. In addition to ZnO NPs use as fertilizers in improving plant growth, yield and Zn biofortification, they can also be promising tool to alleviate environmental stresses, such as salinity and drought ((Dimkpa et al., 2020; Sun et al., 2020). Therefore, in our work, the exposure to low concentration of ZnO NPs and $ZnSO_4$ might play a cellular modulating role. In the contrary, a higher concentration (10 mg mL^{-1}) caused severe physiological and metabolic disturbances. In another study, ZnO NPs at the levels of 400 and 1600 mg L^{-1} increased the germination of cucumber seeds, but became toxic at higher concentrations (De la Rosa et al., 2013). Similar toxicity studies reported that exposure to metal and metal oxide NPs, including ZnO NPs, inhibited seed germination, root elongation and plantlet growth (Lee et al., 2010). In *Arabidopsis thalinana*, the inhibition of seed germination by 400 mg L^{-1} ZnO NPs was shown by Lee et al. (2010).

First hypothesis behind the dual effect of ZnO NPs consists into the controversal effects of Zn ions depending on the concentration. For instance, appropriate amount of Zn as essential micronutrients is needed in several physiological processes in plants (Samreen et al., 2017). Zn ions were also shown to modulate the abundance of proteins related to the antioxidant system, carbohydrate/energy, and amino acid metabolism (Romeo et al., 2014). In our study, indeed, the obtained increase in embryo FM with increasing concentrations of ZnO NPs can be attributed, in part, to the role displayed by Zn in growth modulation, as well as the mitigation of dehydration induced damages, and the improvement of post stress rehydration responses in plant (Upadhyaya et al., 2017).

Taking into account the similar responses of the embryonic axes towards both treatments ZnO NPs and $ZnSO_4$, the mechanism of action of nanosized ZnO NPs seems to exhibit via released Zn^{2+} ions, thereby leading to either positive effects or phytotoxicity, depending on Zn amount (Lee et al., 2010). Zn excess indeed was shown to cause a drastic delay in the growth of seedling, root and shoot in many plant species (De la Rosa et al., 2013).

Alternative possible mechanism of action of ZnO NPs consists into the interference of NPs themselves. Our data suggests that the effects of ZnO NPs can also be attributed to the nanosized particles. This hypothesis was further investigated in order to provide a significant clue about the magnitude of oxidative stress under the exposure to 0.1 mg mL^{-1} (lower concentration) and 10 mg mL^{-1} (higher concentration) of ZnO NPs. The analysis of the antioxidant enzymatic activities revealed that the inhibition of CAT activity by 0.1 mg mL^{-1} ZnO NPs or $ZnSO_4$ suggests the possible role of Zn^{2+} to restrain the oxidative balance at lower concentration. This effect was displayed more significantly with the metallic soluble form of Zn, in comparison with nanosized Zn. In contrary, at higher concentration (10 mg mL^{-1}), CAT activity was enhanced, probably resulting from the activation of the defense response of the stressed embryonic axes in attempt to detoxify the cells and protect the cellular components and molecules. The negative effects of 10 mg mL^{-1} of both treatments on the embryo growth may be associated in major part with the increased induction of oxidative stress. In addition, the changes (stimulation/inhibition) of the antioxidant activities of APX, GPOX and GR in the presence of ZnO NPs in comparison with those occurring in the presence of $ZnSO_4$ revealed, in some part, either the positive/negative interference of Zn^{+2} ions within the enzymatic protein, or else the interference of both Zn ions and nanosized particles.

In literature, the toxic effects of nanosized particles were evidenced to be due to their size, surface area ratio, morphology, nature, composition, reactivity, and others (Zaka et al., 2016; Dimkpa et al., 2020). At cellular and molecular levels, metal/metal oxide NPs can easily enter the cells, and interact with the metabolic processes (Dimkpa et al., 2020). They can induce cellular generation of oxidative stress (Koce et al., 2014) and interfere with the functional groups of biological macromolecules, leading to DNA denaturation, lipid peroxidation, enzymes deactivation, and protein alteration (Koce et al., 2014). In addition, it has been hypothesized that NPs and released ions impede cell metabolism by altering redox status and antioxidative responses (gene expression, enzyme activity) (Koce et al., 2014). Other studies reported the upregulation in the expression of different genes, mainly those involved in defense signaling pathways (Zafar et al., 2016). Higher concentrations of NPs can also promote tissues damage, cytotoxicity, genotoxicity, as well as the inhibition of cell division and ultimately cell death (Zafar et al., 2016).

Furthermore, we inquired into the possible influence of bioavailability, thus the impact of solubility, dissolution and adsorption characteristics of ZnO NPs. Interestingly, SAA data revealed the higher solubility of $ZnSO_4$ compared to ZnO NPs. Hence, the accessibility of Zn ions released from $ZnSO_4$ was higher than ZnO NPs (Tab. 1). This finding suggests that ZnO NPs toxicity might be considered due to the internalization of NPs, which may operate by means of their

physical and chemical properties. Other studies pointed out to a more hamful risk caused by NP on plant species than their bulk counterparts or their soluble metallic ions.

Additionally, the determination of other micronutrients (Mn, Zn, Fe and Cu) in the embryonic axes suggests that bioavailability of ZnO NPs is associated with many factors related to the NPs themselves or to Zn ions. Besides, the intracellular Zn homeostasis could be affected through Zn influx, efflux, translocation, accumulation and intracellular compartmentalization. Moreover, in this study, we evidenced antagonistic relationships between Zn and some nutrient elements of two-capacity cations, mainly Fe. Indeed, Fe showed competitive behavior with Zn in the presence of higher concentrations of ZnO NPs and $ZnSO_4$, however Mn and Cu contents were not significantly changed with Zn increase. This may lead to changes within the accessibility of the essential microelements to the embryonic cells, which can result in changes of the metabolic and physiological balance by local competition in different places. In other studies, it was indeed reported that Zn has a high chemical similarity to Fe, therefore it can substitute for this metal ion in the active sites of enzymes, and consequently interfere with cellular functions (Zargar et al., 2015). For example, Fe is involved in the activation of many metabolic, physiological and biochemical pathways in plants, and it serves as a prosthetic group constituent of many enzymes (Rout and Sahoo, 2015). Besides, ZnO NPs were able to interrupt the apoplastic and symplastic pathways (Yahyaoui et al., 2017), thus inhibiting the process of absorption of water and micro- and oligoelements such as Fe and Mn (Dimkpa et al., 2014).

Conclusion

Our study may bring novel insight at the potential application of Zinc based nanofertilizers to efficiently correct Zinc deficiency and to enhance plant growth. In addition, we could reveal more understanding of the physiological mechanisms of ZnO NPs to improve early growth of *Lathyrus* seedlings. We suggest either the dissolution of Zinc ions from ZnO NPs and their interference with the metabolic pathways in plant system. However, for *Lathyrus*, it appears that ZnO NPs were benefecial when applied at low levels, but became toxic at higher concentrations. Hence, in attempts to avoid NPs toxicity, further analyses are needed to elucidate their response depending factors, such as applied concentration, plant species, stage of development and culture condition.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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