Zincum Metallicum an Homeopathic Drug Alleviates Zn-Induced Toxic Effects, Promotes Plant Growth and Antioxidants Capacity in *Lepidium Sativum*

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Abstract

In this study, we investigated the effect of Zincum Metallicum (ZM) on zinc (Zn) toxicity in the plant species *Lepidium sativum.* We focused on growth, Zn uptake and numerous biochemical parameters. Seedlings were hydroponically subjected during 7 days to 0.05, 500, 1000, 1500 and 2000 µM Zn^{2+}, in the absence or presence of 15ch or 9ch ZM. In the absence of ZM, Zn induced negative effect on growth especially at the dose of 2mM. Zn induced also chlorosis, reduced total chlorophyll and/or carotenoid content and increased the level of malondialdehyde (MDA). Under Zn-toxicity (500, 1000 and 1500 µM), the superoxide dismutase (SOD), catalase (CAT), gaiacol peroxidase (GPX) and glutathione reductase (GR) activities were increased and/or unaltered, while at 2000 µM Zn affected the activity of these enzymes. At the highest Zn level (2 mM), proline and total polyphenols and flavonoids contents were markedly induced in leaves and roots of *L. sativum.* Additionally, the supply of ZM in the nutrient medium considerably ameliorated the plant growth, photosynthetic pigments and the studied non-enzymatic antioxidant molecules and enzymatic activities against Zn induced-oxidative stress. Our data suggest that the potential homeopathy properties of ZM may be efficiently involved in the restriction of Zn-induced oxidative damages, by lowering Zn accumulation and translocation in the leaves and roots of *Lepidium sativum*.

Introduction

Zinc (Zn) is an indispensable micronutrient for growth and development of plants, but it is considered as a major mineral pollutant (Li et al. 2013). Under normal conditions, Zn is required in small amount to allow the important plant physiological, biochemical and molecular pathways including photosynthesis, nitrogen metabolism, chlorophyll and auxin synthesis, activation of enzymes (cofactors), DNA replication and transcription, and the control of gene expression (Rout and Das 2003). It plays a key role in the maintain of membrane integrity and permeability, the protection of thiol groups of proteins and the inhibition of reactive oxygen species production (ROS) induced by transition metals including iron and copper (Haleng et al. 2007). However, elevated Zn concentrations might inhibit plant growth by affecting plant physiological metabolisms (Tsonev et al. 2012). In this circumstance, substantial data revealed that high Zn concentrations inhibited cell division and cell elongation of roots system, decreased photosynthesis, disturbed mitochondrial structure and absorption and translocation of nutrients and induced the overproduction of ROS (Tsonev et al. 2012; Rout and Das 2003, Doillan, 2010). Excessive production of ROS in plant would, therefore, enhance oxidative stress and disrupt the redox homeostasis (Todeschini et al. 2011). For these reasons, many previous studies focused on elucidating the negative impacts of heavy metals on plant crops and improving their growth and productivity (Tsonev et al. 2012; Li et al. 2012; Dos Santos et al. 2019).

The application of homoeopathy in agriculture, known as agricultural homoeopathy, would be very effective in terms of plant tolerance to abiotic stress, particularly heavy metals. In fact, numerous studies have shown that potentiated homeopathic medicine can improve the physiological activities in numerous cultivated plants species, via modulating enzymatic activities, total sugar, protein and chlorophyll content

(Mazón-Suástegui et al. 2019).For instance, the study of Banerjee P and Sukul NC. (2013). revealed that cuprum sulphuricum, anhomeopathic drug, alleviated toxic effect of copper though promoting seed germination and peroxidase activity in *Vignaun guiculata*

Garden cress (*Lepidium sativm* L.), an annual herbaceous species from the *Brassicaceae* family, is one of the well-known aromatic and medicinal plant (Vaishaliet al. 2014). It is of economic importance since it has been used as treatment of various diseases and the reduction of the effects of chemotherapy (Abo El Maatiet al. 2016). *Lepidium sativm* is a rich in bioactive molecules, including fatty acids, tocopherol, carotenoid, phytosterol, campesterol, avenasteroland phenolic compounds, to which the antimicrobial, antihypertensive, antioxidant, antispasmodic, antidiarrhoeal, antiasthmatic, hypoglycaemic and hypolipidemic activities are attributed (Abo El Maati et al. 2016). Despite the fact that *L. sativum*have been studied for its tolerance to abiotic stress including salinity (AlSammarraie et al. 2020) and copper excess (Rombel-Bryzeket al. 2017), little is known about its response to zinc (Zn) contrast.

The current study aims to explore the effect of different Zn concentrations on the growth, physiological and biochemical parameters of *L. sativum* and evaluate the putative effect of the exogenous application of Zincum Metallicum (15 or 9 ch) on Zn-induced toxicity. We focused essentially on the growth parameters, chlorophyll, carotenoids content proline contents, lipid peroxidation and antioxidant system.

Material And Methods

Plant Materiel and growth conditions

Seeds of *L. Sativum* were collected from the region of Djerba (33° 48' 27.353" N 10° 50' 42.529" E). Seeds were disinfected and germinated in Petri dishes containing two sheets of filter paper soaked with distilled water. Five days after germination, seedlings were transplanted into plastic pots containing 1.3 L aerated and renewed quarter-strength Hoagland nutrient solution (Hoagland and Arnon1940).Plant culture was maintained in a growth chamber under 22°C, 8 h of photoperiod per day and a relative humidity of 86 %.Three weeks after, individual plants were exposed to 0.05 (control), 0.5, 1, 1.5 and 2 mM of zinc, in the form of $ZnSO_4$, added or not with two concentrations (9 or 15 ch: Hahnemannian centesimal) of Zincum Metallicum(ZM) for 7 days prior to the final harvest. At the harvest, fresh weight of roots and leaves were separately recorded for six plants randomly selected from each treatment and used for physiological analysis. Roots and leaves were also harvested from three plants of each treatment and frozen at-80°C or air dried for the biochemical analysis.

Plant growth parameters and water content

The weight of leaf and root samples were measured before (fresh weight: FW) and after (dry weight: DW) dryness at 60 °C for 3 days. Water content (WC) was determined as:

WC = (FW - DW)/FW x 100

Tissue zinc content

Zinc was extracted from 25 mg dried material transferred to 125 mL conical digestion flasks. Twelve (12) mL of triacid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1 (v/v)) were added to the flasks. Plant materials were digested in cold for 3 h followed by digestion for 2–3 h on a hot plate, until the digest was clear or colorless. The flasks were allowed to cool and the contents were diluted to an appropriate volume (Sahraw at and al, 2002).then assayed by atomic absorption spectrophotometer (Perkin Elmer Analyst 300), using standard with known concentrations.

Chlorophyll and carotenoids content

Chlorophyll (Chl) and carotenoids was extracted from 5 mg of fresh leaf tissue using 25mMTris-HCL (pH 7.6), 1 mM EDTA, 1mM $MgCl_2$and 14 mM β-mercaptoethanol.50 µl of the extract were taken and then homogenized in 1450 µl of 80% acetone. The mixture is stored at 4°C over night. The samples were centrifuged for 15 min at 1500 gand the supernatant was used to identify absorbance of chlorophyll a and b, and carotenoids at 645, 663 and 440.5nm, respectively(Marker and al 1980). Total chlorophyll and carotenoids content (mg g^{-1} FW) were calculated according the following formula:

Chl a = (12.7×DO663)-(2.69×DO645) ×v/(w×1000)

Chl b = (22.9×DO645)-(4.68×DO663)×v/(w×1000)

Chl tot=(20.2×DO645)-(8.02×DO633) ×v/(w×1000)

Carotenoids =46.95×(DO440.5-0.268×Chl a+b)

Malondialdehyde determination

Oxidative damage in *L. Sativum* was estimated based on malondialdehyde (MDA) content, which was determined from fresh leaf and root tissue, following the method of Draper et al. 1990. One hundred milligrams were ground in 1 mL of 0.1% trichloroaceticacid at 4°C. After centrifugation at 15000 g for 15 min, a250µl aliquot of supernatant was added to 1ml thiobarbituric acid (prepared in 20% trichloroacetic acid) and heated for 30 min in a water bath at 95°C. Samples were again centrifuged at 10000g for 10 min and the absorbance of the supernatant was measured at 532 nm. After subtracting the nonspecific absorbance at 600 nm, MDA concentration (mol g^{-1} FW) was determined using a molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Proline content

Proline content was measured by the method of Bates and al. (1973). Fresh leaves and roots (25mg) were mixed with 1 ml of sulfosalicylic acid (3%). The samples were centrifuguated at 12.000 g for 20 min. A 500 µl aliquot of supernatant were added into 500 µl of sulphosalicylic acid, 1 ml of concentrated acetic acid and 1 ml of ninhydrin. After incubation at 100°C for 1 h, the reaction was

stopped by placing the test tube in an ice bath. Two milliliters of toluene were added to the solutions. The organic toluene phase was separated and used to determine the content of proline at 520 nm.

Protein and antioxidants enzyme assays

Fresh plant material (200 mg) was extracted in a buffer containing Potassium hydrogen phosphate, EDTA, PVP and glycerol. The homogenate of each sample was centrifuged at 12.000 rpm for 10min. The supernatant fraction was then assayed for proteins and various antioxidant enzymes.

Proteins contents were analyzed according to the method of Bradford (1976), using the principle of Coomassie G250 blue binding with proteins. The concentration of protein was determined from a standard range of BSA (Bovine Serum Albumin) between 0 and 10 μgmL^{-1}.

The Superoxide dismutase (SOD) activity was determined by using the tetrazolium nitroblue (NBT) / riboflavin according to the method of Beauchamp and Fridovich (1971).Briefly, the samples mixture containing phosphate buffer 50 mM, (pH 7.8), EDTA 0.1 mM, L-methionine 13 mM, riboflavin 2 µM and NBT 75 µM. The reaction was initiated by exposing the reaction mixture for 15 min to a 50 $\mu mol.m^{-2}.s^{-1}$ fluorescent light source. The absorbance was spectrophotometry measured at 560 nm.

The Catalase (CAT) activity was determined spectometrically according to the method of Chaparro-Giraldo et al (2000), by measuring the disappearance rate of hydrogen peroxide (H_2O_2) at 240 nm. The reaction mixture contained potassium phosphate buffer (50nM, pH7), 100µl of enzyme extract and H_2O_2 at 30%.

The Glutathione peroxidase (GPx) converts H_2O_2into water (H_2O) by the transformation of Glutathion (GSH) to Glutathione disulfide (GSSG).Activity of GPx was assayed by measuring the disappearance rate of H_2O_2according to the method of Flohe and Gunzler, (1984).This activity was expressed in µmol of GSH/min/mg of protein.

The Glutathione reductase (GR) activity was determined by monitoring the oxidation rate of NADPH at 340 nm (Rao et al. 1996). The reaction mixture (1 ml) contained 100 mM phosphate buffer (pH 7.8), 2 mM EDTA, 0.5 mM oxidised glutathione, 0.5 mM NADPH, 0.2 mM NADPH and the reaction was initiated by adding 100µl of the enzyme extract.

Extraction and determination of total polyphenols compounds

Roots and leaves were air-dried at room temperature for 2 weeks. Samples extracts were obtained by magnetic stirring of 1 g of dry powder per sample in methanol 80% (10 ml)) for 30 min and kept at 4 °Cfor 24 h. The methanolic extracts obtained were filtered through a Whatman filter paper (N°.4) and stored at 4 °C.Colorimetric quantification of the total phenolic compound was conducted using the Foline-Ciocalteu reagent, as described by Dewanto et al. (2002). Briefly, an aliquot of 125 µl of 1/10 diluted sample from each methanolic extract was dissolved in 500 µl distilled water and 125 µl Folin–Ciocalteu

reagent. After shaking and resting the mixture for 3min, 1250 µl of 7% Na_2CO_3 was added to the mixture and adjusted with distilled water to a final volume of 3 ml. The mixture was then incubated for 90 min at room temperature in the dark. The absorbance was read at 760 nm and the total phenolic compound was expressed as milligram gallic acid equivalent per gram of dry weight (mg GAE g^{-1} DW) through the calibration curve of gallic acid (0-500 mg l^{-1}).

Determination of total flavonoids content

The total flavonoid content was determined by using a colorimetric method described previously (Zhihenet al.1999). An aliquot (75 µl) of 7% sodium nitrite ($NaNO_2$)solution was added to each extract (250 µl). The mixture was shaken for 6 min before adding 0.15 µl of 10% aluminum chloride ($AlCl_3$). After 5 min, 0.5 ml of 1M sodium hydroxide (NaOH) was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm. Total flavonoid was expressed as milligram catechin equivalent per gram dry weight (mg CE g^{-1} DW) using a calibration curve developed for catechin (0-500 mg l^{-1}).

Statistical analysis

Data were subjected to a one-way ANOVA test using ANOVA, CoStat software, version 6.4, CoHortSoftware, Monterey, CAand means were compared according to Duncan's multiple-range test at 5 % level of significance.

Results

Plant growth and Water content

The effect of Zn stress on the morphology of *L. sativm* plants were marked by a decrease of shoot length in plants subjected to all Zn concentrations (0.5, 1, 1.5 and 2 m M) for 7 days (figure1).Leaf and root biomass were decreased with increasing Zn concentration in the medium culture. In the leaves, these decreases reached 80% and 72%respectively under 1.5 and 2 mM Zn2+. The same variation was recorded for the roots (Table 1).Zinc treatment significantly reduced tissue hydration of leaves and roots. This effect was more pronounced in the shoots since the water content dropped from 92 % to 84% upon treatment with2000µM of Zn (Table 1).

The addition of ZM to Zn-treated plants alleviated significantly the Zn-induced growth reduction in *Lepidium sativum* L. Hence, plant subjected concomitantly to Zn and ZM produced significantly more fresh leaves as compared to their respective ones subjected to Zn alone (Table 1). This effect was more pronounced with the ZN 15 ch as compared to ZM 9 ch. For example, in plant subjected to 1.5 mM Zn, addition of ZM 15 ch increased 3 times the leaf biomass (Table 1). For the WC, the addition of ZM to the Zn-containing medium does not affect this parameter in both organs (Table 1).

Zinc accumulation

The concentration of Zn in the shoots and the roots of *L. sativum* plants increased with increasing the concentration of this metal in the nutrient solution (Figure 2). For all Zn concentrations, roots accumulated more Zn than the shoots. Hence, Zn content reached 60000 µg Zn /gDW in roots of plants exposed to 2000 µM Zn, but that of the leaves does not exceed 40000 µg/g DW, for the same Zn dose. The addition of ZM to the nutrient solution decreased significantly Zn accumulation in leaves and roots compared with Zn treatment alone. For instance, Zn concentrations in leaves and roots of ZM+Zn treated plants were reduced by 20 and 11%respectively, compared to Zn-treated plants (Figure 2). We showed also that, in the leaves, ZM 15 ch reduced more intensively the Zn accumulation than ZM9ch, but in the roots both ZM concentrations induced the same effect.

Chlorophyll and carotenoids content

Data illustrated in Table 2 showed that 1000, 1500 and 2000 µM Zn significantly reduced the content of total chlorophyll. In particular, the application of 2000 µM Zn caused up to 80% reduction of this pigment concentration in the leaves. On the other hand, the concentrations 1500 and 2000µM Zn resulted in a marked reduction of carotenoids; while 500 and 1000µM Zn enhanced the content of these pigments (Table 2). Zincum Metallicum at doses of 9 or 15chsupplied to the culture mediumled to a significant increase in the level of these pigments, relative to plant treated only with Zn alone.

MDA content

The increase in the Zn concentration in the medium induced increasing MDA content in the leaves and roots of *L. sativium* plants. As compared to control plants, the highest dose of Zn (2000µM) increased MDA levels up 6 and 3.5 times in leaves and roots, respectively (Figure 2). In the presence of µM of Zn, addition of ZM increased the MDA in the shoots and roots. The supply of ZM to Zn-stressed plants reduced leaves and roots MDA content, compared to those treated only with Zn. For both organs (root and shoot), the dose 15 ch of ZM induced more important MDA reduction compared to that induced by the dose 9 ch. (Figure 3).

Proline accumulation

The free proline concentration in tissues was positively correlated with the Zn concentration in the medium(Figure 4).For instance, this amino acid concentration was increasedby7 and 5 times in leaves and roots of plant treated with 2000 µM Zn, respectively, compared to that of the control (0.05 µM Zn). The addition of ZM, at the doses 9 or15 ch, further enhanced the content of proline, to reach up to 56 and 31%in leaves and roots of plants treated with 2000 µM Zn, respectively (Figure 4).

Under control condition of Zn, the addition of ZM enhanced the accumulation of proline in the leaves and in the roots.

Enzymes activities

Data presented in Figure 5 and 6 showed that the activities of SOD, CAT, GPX and GR varied to a different extend depending on Zn and ZM concentrations and the plant organ. As compared to that in control plants (0.05 µM Zn), the activity of SOD in the leaves was not significantly altered in Zn-treated plants. Whereas it was significantly increased in roots of Zn-treated plants with 500, 1000 and 1500 and it was dropped in the roots of at 2000 µM Zn-treated plants (Figure 5a and b). In plant cultivated under 0.05 µM Zn, the addition of ZM induced light and not significant increase in SOD activity in leaves and significant increase only at 15 ch.

CAT activity was significantly increased in both leaves and roots of plant treated with 500, 1000 and 1500, except that in roots of 500 µM Zn-treated. Nevertheless, the activity of this enzyme was dropped by 20% and 66% in both leaves and roots of plants treated with 2000 µM Zn, respectively (Figure 5 c and d).GPX and GR activities were increased or maintained stable across all the Zn treatments (Figure 5 a and b). At equal Zn concentrations, the leaves and roots of ZM-Zn treated plants showed highest activities of the above-mentioned enzymes than those of the Zn alone-treated plants.

Accumulation of phenolic compounds

Similarly to the antioxidant enzymes activities, the total polyphenols and flavonoids contents varied to a different extend depending on Zn concentration and treatment as well as plant organ. The total polyphenols content was not significantly altered in leaves and roots of plants treated with Zn alone for all used doses, except that of roots treated with 1500 and 2000 µM (Table 3). The flavonoids content significantly increased in leaves and roots of plants treated with 1000 1500 and 2000 µM Zn (Table 3). Under adequate zinc concentration in the medium (0.05 µM), the addition of ZM, at 9 and 15 ch, not induced significant changes in total polyphenls and flavonoids in the roots and the leaves of *L. sativium* plants. The combined treatment of Zn with ZM increased the total polyphenol and flavonoids levels in both organs compared to that treated with Zn alone, mainly in the presence of 2000 µM and the ZM dose at 15 ch (Table 3).

Discussion

Garden cress (*Lepidium sativum* L.),is one of the edible medicinal plant in Tunisia, providing an important source of proteins, carbohydrates, dietary fibers and minerals including calcium, phosphorus, potassium, zinc (Manohar et al. 2012, Alqahtani et al. 2019).

In the current study, we focused on improving zinc tolerance of this species to increase its agricultural production. The study evaluated, for the first time, homoeopathic drug, Zincum Metallicum (ZM), to alleviate Zn-induced stresses in this species. Indeed, the results showed that fresh weight of *L. sativum* plants was significantly reduced after exposure to 1000 µM Zn alone with the appearance of necrotic symptoms in leaves. This decrease was markedly pronounced in plants grown under 2000 µM Zn. This suggests that, under these doses, *L. sativum* is sensitive to Zn excess. Hence, as consequence of Zn treatment, water content were significantly reduced, mainly in leaves, suggesting that Zn induced tissue dehydration. In a previous study, the fresh weight of *pisum sativum* was significantly reduced at different

Zn concentrations (35, 70, 350, 700 µM) (Stoyanova and Doncheva, 2002), showing signs of toxicity essentially leaf chlorosis. In the study of kastori and al. (2008), the relative water content was significantly decreased in sunflower plants subjected to Zn- excess.

According to our study, the uptake of Zn increased in leaves and roots with increasing Zn concentration in the medium. We showed also that Zn accumulation was more than 98 % in roots compared to leaves. These results suggest that *L. sativum* adopts root accumulation of Zn^{2+} as solution to prevent its transport to the photosynthetic organs.

Reductions in plant growth due to heavy metals accumulation are often associated with enhanced in pigments degradation. In *L. sativum*, a significant decline in its total chlorophyll levels was noticed in plants subjected to 1000, 1500 and 2000 µM Zn concentrations. Similar results were reported by Samreen et al. 2017 in different varieties of mung beans treated with 2 µM of Zn. The Zn toxicity may be induced by either the decrease in chlorophyll synthesis or the increase in its degradation or to the inhibition of photosynthetic electron transport (Vaillant et al. 2005). Carotenoids content was markedly increased under exposure to 500 and 1000 µM Zn, suggesting that this metabolite was less sensitive to the Zn treatment. Under mercury exposure, Smolinska et al. (2017) also reported that carotenoid content increased in leaves of *L. sativum* plants.

The exposure of plants to heavy metals resulted often from the inevitable oxidative stress, considered as the main factor responsible for membrane cell damage (Smeets et al. 2008). It has been demonstrated that oxidative stress in *L. sativum* was induced by different trace elements including mercury (Smolinska and al. 2017), copper (Rombel-Bryzek et al. 2017) and arsenic (Umar et al. 2013) and other abiotic stressors like salinity, drought, temperature and light exposer (AlSammarraie and al. 2020). Under such constraints, the effects of oxidative stress on membrane peroxidation are often estimated by determining the content of MDA, which is used as an indicator of membrane lipid peroxidation. The results obtained in this study revealed that elevated zinc concentrations (1500 and 2000µM) increased MDA content in the leaves and roots of *L. sativum* plants. This effect can be explained by the overproduction of ROS and/or the disturbance of the antioxidant system inducing hence lipid-membrane peroxidation as demonstrated by Chaoui et al. (1997). It has been demonstrated that Zn induced acute oxidative stress in *Triticumae stivum* (Khan et al. 2007).Besides; this phenomenon can be initiated by the iron-containing enzyme lipoxygenase, a membrane-bound enzyme, which is known to oxidize polyunsaturated fatty acids and to produce free radicals, mediating thus lipid peroxidation (Chaoui and al. 1997).

To cope with oxidative stress, the plant activates its antioxidant defense system, which consists of osmoticum, enzymes and antioxidant molecules. For instance, the proline kwon for its role as an osmoticum, would act as scavenging agent of ROS including singlet oxygen ($_1O^2$) and hydroxyl radical (OH^{-}.), thus ensuring the protection of cellular structure and functioning (Sharmila and PardhaSaradhi, 2002).The results of our study revealed that Zn significantly increased accumulation of proline in plants treated with zinc. In the same context, the study of Li et al.2013 reported that constitutive osmotic

regulator concentrations including proline, total soluble proteins and total soluble sugars were enhanced in the leaves of wheat plants treated with zinc.

On the others hand, our results revealed that Zn(500, 1000 and 1500 µM) stimulated the roots activity of SOD, while that of leaves was unchanged across all the Zn treatment. SOD stimulation is also observed in many plants when subjected to Zn-metallic constraint, such as beans (Michael and krishnaswamy, 2011). Catalase is an enzyme that catalyses the decomposition of H_2O_2 into oxygen and H_2O. The treatment of plants with zinc induces a more or less significant increase in catalase activity both in the leaves and in the roots. In a previous study, Jain et al. (2010) revealed that Zn stimulated CAT in sugar cane. The increase in the activity of this enzyme highlightits important role in the detoxification of H_2O_2 in Zn-treated plants (Prasad et al., 1999). Although, in this study we revealed that CAT activity was inhibited by 2000µM Zn, suggesting the incapacity of *L. sativum* to tolerate high concentration of Zn. In addition, the activity of GPX and GR activities were increased or maintained stable across all the Zn treatments glutathione peroxidase (GPX)was stimulated or maintained unchanged across all the Zn treatment. These results confirm those obtained in the study carried out by Duman and Ozturk (2010) on *Nasturtium officinale* treated with increasing doses of Nickel. The induction of GR after zinc treatment has also been reported by Gupta et al, (2011). The data on the variation in GR activity under metal treatment are quite contrasted. Indeed, some studies show its induction by the metal treatment, while others reveal an inhibitory effect on the activity of the enzyme. Indeed, the induction of GR activity can be attributed to an increase in its substrate (GSSG: oxidised form of glutathione) (Lin et al. 2007). The results obtained are in line with the work carried out on *Hibiscus cannabinus* by (Feng et al., 2013) where he reported that the glutathione reductase activity (GR) was much larger than the control, ensuring that sufficient quantities of GSH were available to respond to the cadmium stress. In barley, Finkemeier et al (2003) observed a 50% reduction in GR activity under cadmium treatment. Inhibition of this activity after treatment with cadmium or copper was also reported by Braha et al., 2007.

Besides, the current study revealed that Zn enhanced the accumulation of polyphenols and flavonoids in the leaves and roots of *L. sativum*. This accumulation is more marked in the leaves than in the roots. This endogenous increase can be attributed to their potential antioxidant and their ability to eliminate ROS (Achat, 2013).

To alleviated Zn-induced toxicity in *L. sativum*, the homoeopatic drug, ZM, was assessed in plant treated with different Zn concentrations (500, 1000, 1500 and 2000). Results showed that ZM alleviated significantly Zn-induced phyto-toxic effect. The improvement concerned the growth parameters and manifested by an increases in the fresh biomass and in the content of photosynthetic pigments concomitant to a reduction in the accumulation of Zn^{2+}especially in the leaves, in plants treated with the combination of zinc and ZM compared to the plant treated only by zinc. The antioxidant enzymes activity: superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and glutathione reductase (GR), are increased in the presence of zinc. However this stimulation was more important in the presence of homeopathy. In addition, to the antioxidative enzymes, other molecules could play important roles in the alleviation of zinc toxicity in *Lepidium sativum* as proline, polyphenols and flavonoids. In fact,

the biosynthesis of these compounds was stimulated by Zn and this overproduction was more important under combined treatment zinc-homeopathy .Similar results were reported by Mazón-Suástegui (2019), where the authors revealed that agricultural homoeopathy is able to stimulate favorable biological and even genetic responses in *Ocimum basilicum*, *Phaseolus vulgaris*, *Cucumis sativus*, and *Solanum lycopersicum* growing under different abiotic stress conditions. In addition, Banerjee et al. (2013) reported that homeopathic compounds might reduce the effect of copper toxicity by promoting seed germination and peroxidase activity in *Vigna unguiculata*

Conclusion

In summary, our findings showed high sensitivity of *Lepidium sativum* to zinc stress as indicated by the reduced growth of leaf and root, enhanced zinc ions accumulation, degradation of photosynthetic pigments and amplified level of malondialdehyde (MDA). The increase of the anti-oxidative enzymes activities and content of proline, total polyphenols and flavonoids, by Zn stress, were however unable to restore the morpho-physiological parameters, to inhibit lipid peroxidation and restrict the accumulation of Zn^{2+} in leaves and roots. The ZM supply with Zn treatment reduced the toxicity symptoms of this metal to some extent. In fact, the ameliorative effect of this homeopathic drug on plant biomass production was paralleled by the restoration of the pigments amounts and the inhibition of MDA content along to higher production of proline and polyphenols and an improvement of the antioxidant enzymes activities (SOD, CAT, GPx and GR). Our obtained results indicate that the presence of ZM mainly at the dose 15 ch affects different physiological and biochemical aspects in *Lepidium sativum* plants in response to zinc stress and this could be recognized as an adaptive mechanism against heavy metal constraints.

Abbreviations

Zinc (Zn); Hydrogen peroxide (H_2O_2);Reactive oxygen species (ROS); Malondialdehyde (MDA); Superoxide dismutase (SOD);Catalase (CAT); Glutathione peroxidase (GPx);Glutathione reductase (GR); Zincum Metallicum (ZM);

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials:

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Authors' contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ghazwa BOUDALI. Conceptualization was made by Chiraz CHAFFEI-HAOUARI .The first draft of the manuscript was written by Ghazwa BOUDALI, Saoussen BEN-ABDALLAH and Tahar GHNAYA and all authors commented on previous versions of the manuscript. Formal analysis was made by Abdala CHALAH. Zeineb OUIRGHI is the director of the laboratory and Abdelaziz SEBEI helped as in the mineral analysis. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, table 1-3 is only available as a download in the Supplemental Files section.

Figures

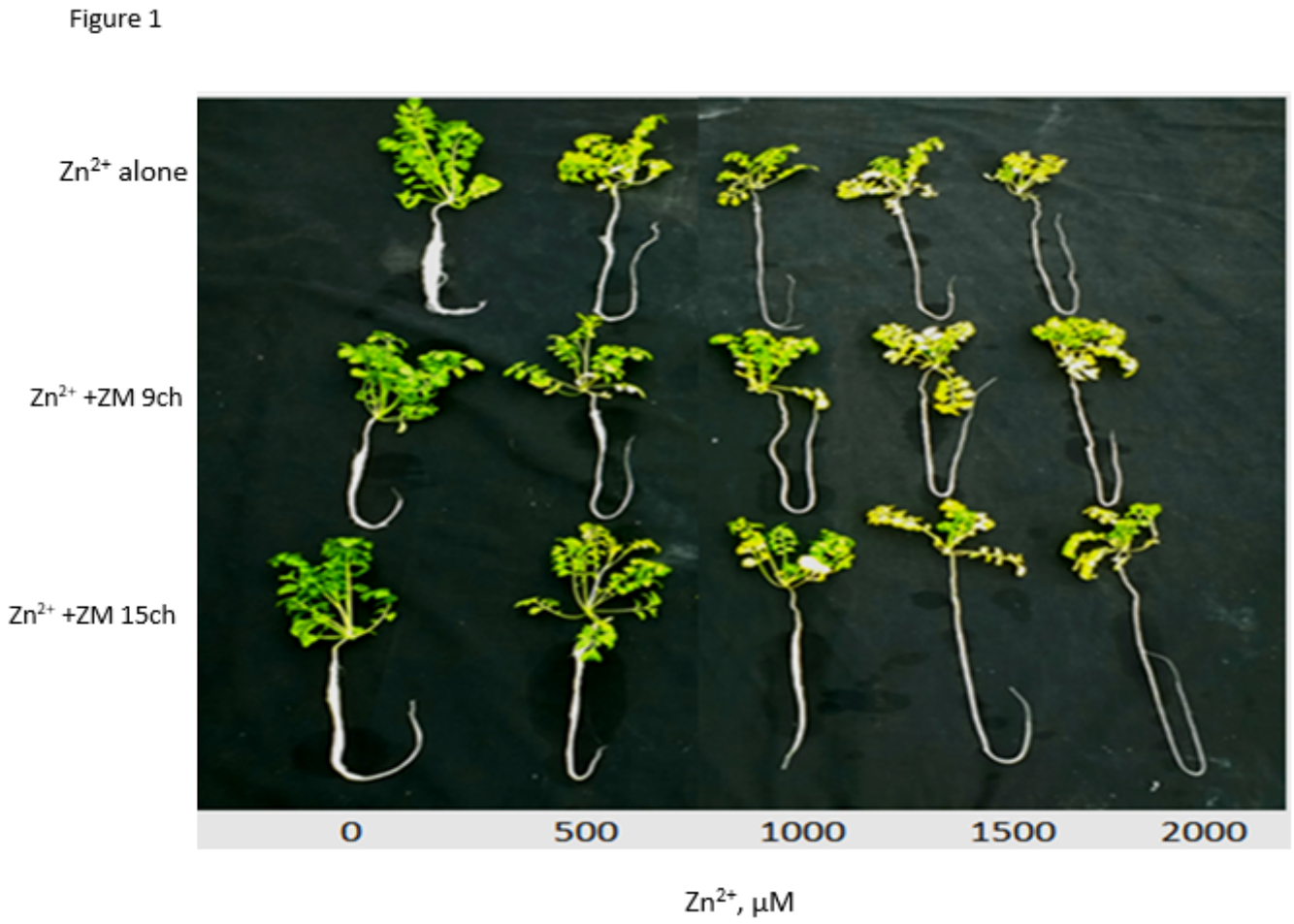

Figure 1

Phenotype of Lepidium sativm grown under different Zinc (0, 500, 1000, 1500 and 2000 µM) concentrations or in combination with zincummetallicum(9 or 15 ch)for 7 days.

Figure 2

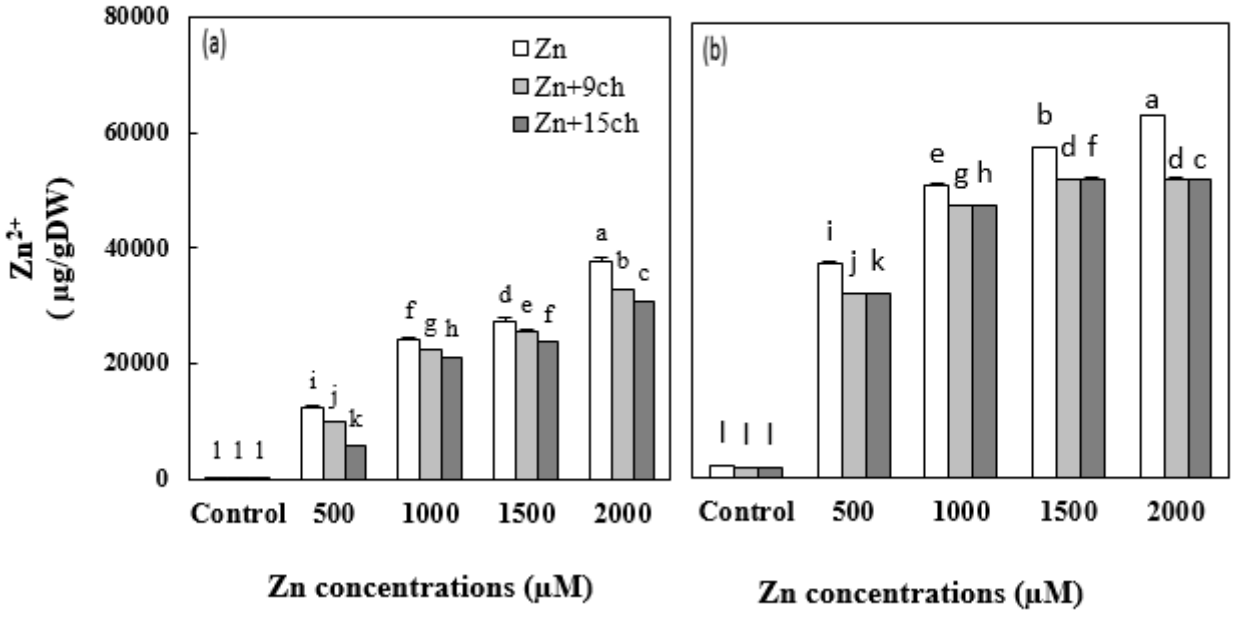

Figure 2

Zinc content of Lepidium sativm grown in 0, 500,1000, 1500 and 2000 µM Zn alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days(a: leaf ;b: root). Means of six replicates ±standard error. Values followed by different letters are significantly different according to Duncan's multiple-range test at 5 %.

Figure 3

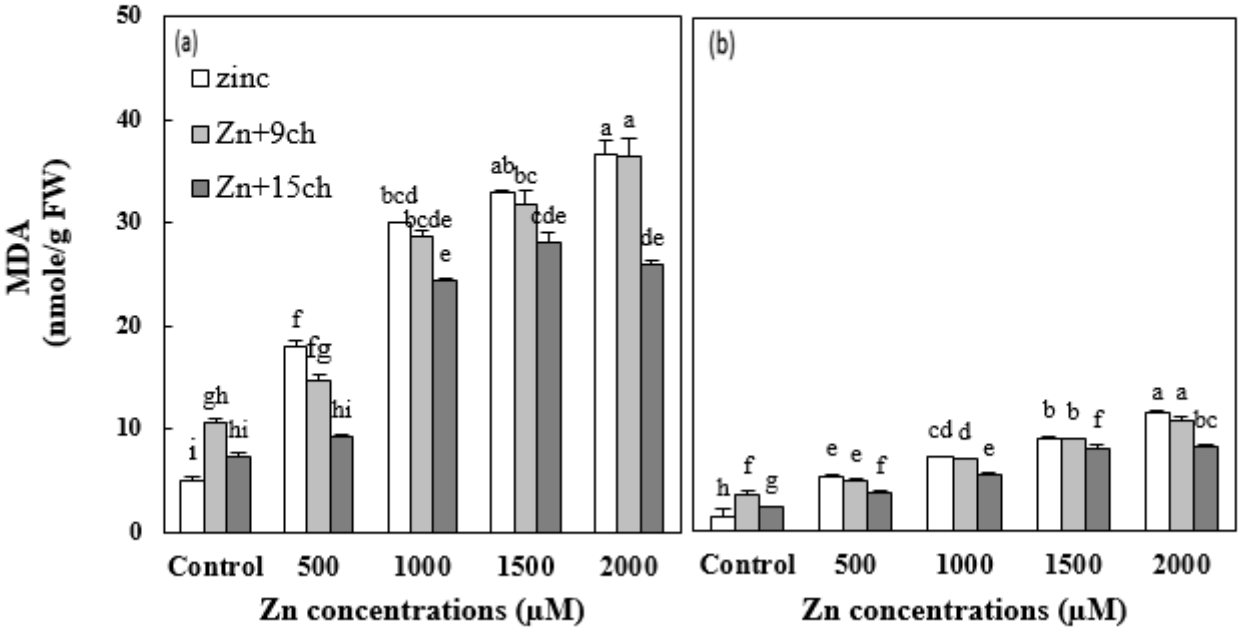

Figure 3

MDA content of Lepidium sativm grown in 0, 500,1000, 1500 and 2000 µM Zn alone or in combination with Zincum Metallicum(9 or 15 ch)over 7 days(a: leaf ;b: root). Means of six replicates ±standard error.Values followed by different letters are significantly different according to Duncan's multiple-range test at 5 %.

Figure 4

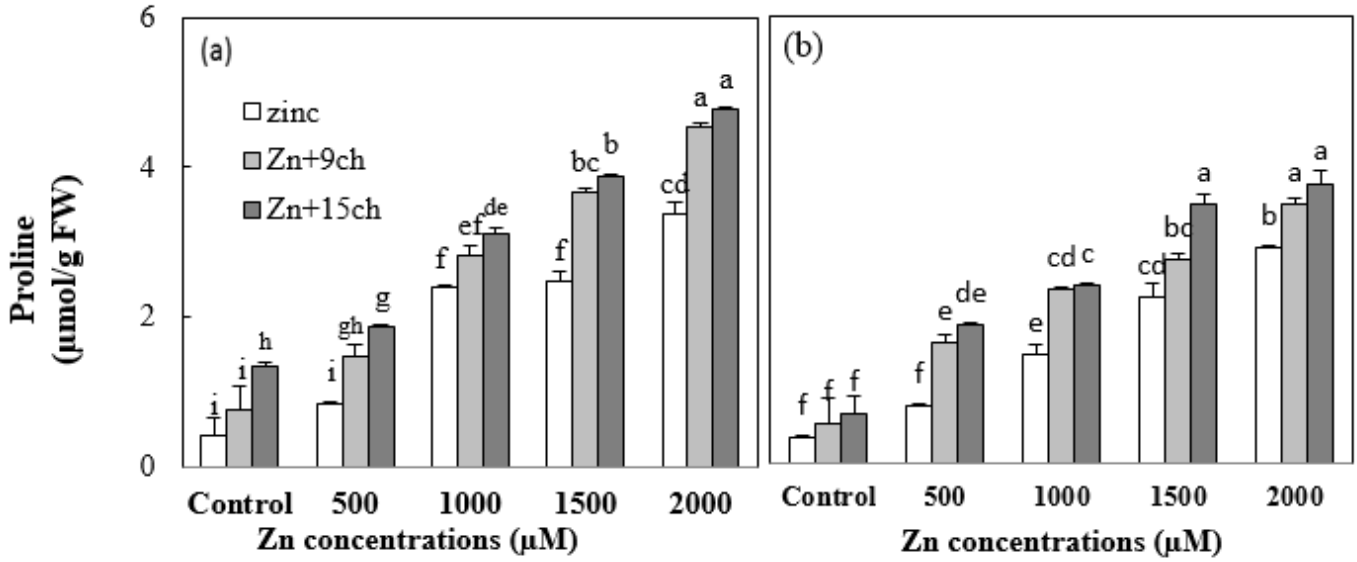

Figure 4

Proline content of Lepidium sativm grown in 0, 500,1000, 1500 and 2000 µM Zn alone or in combination with Zincum Metallicum(9 or 15 ch)over 7 days(a: leaf ;b: root). Means of six replicates ±standard error.Values followed by different letters are significantly different according to Duncan's multiple-range test at 5 %.

Figure 5

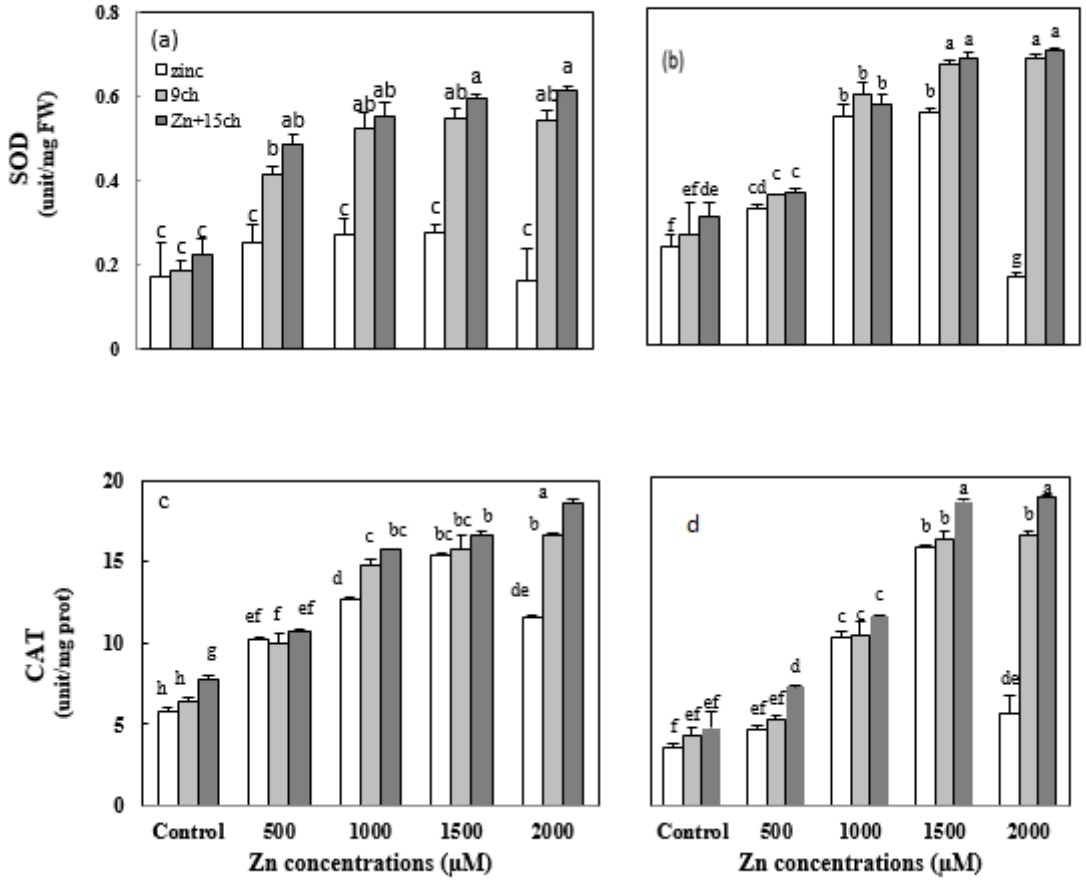

Figure 5

Superoxide dismutase and catalase activitiesin leaves (a and crespectively) and roots (b and d respectively) of Lepidium sativm grown in 0, 500,1000, 1500 and 2000 μMzincalone or in combination with zincummetallicum(9 or 15 ch)over 7 days. Means of six replicates ± standard error.Values followed by different letters are significantly different according to Duncan's multiple-range test at 5 %.

Figure 6

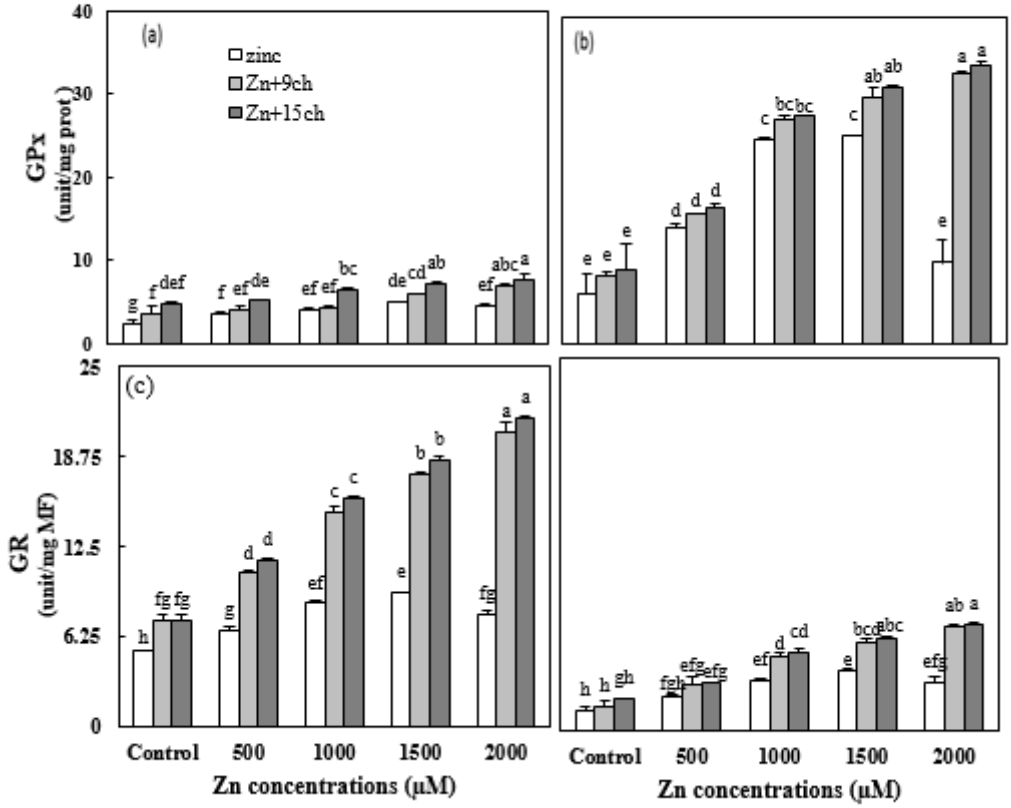

Figure 6

Glutathione peroxidase and glutathione reductase activitiesin leaves (a and crespectively) and root (b and d respectively) of Lepidium sativm grown in 0, 500,1000, 1500 and 2000 µMzincalone or in combination with zincummetallicum(9 or 15 ch)over 7 days. Means of six replicates ±standard error.Values followed by different letters are significantly different according to Duncan's multiple-range test at 5 %.

Supplementary Files

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