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[1]Laboratory of Legumes and Sustainable Agrosystems, Centre of Biotechnology of Borj-Cedria, Hammam-Lif, Tunisia
[2]Faculty of Sciences of Gabès, University of Gabes, Gabes, Tunisia
[3]Laboratory of Extremophiles Plants, Centre of Biotechnology of Borj-Cedria, Hammam-Lif, Tunisia

Effect of increasing zinc levels on *Trigonella foenum-graecum* growth and photosynthesis activity

Fadwa Melki[1,2], Ons Talbi Zribi[3], Sabrine Jeder[1,2], Faten Louati[1], Issam Nouairi[1], Haythem Mhadhbi[1], Kais Zribi[1*]

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Summary

Zinc is an indispensable element for the plant growth and the cellular metabolism. However, this mineral element becomes harmful at high quantities. The effects of high zinc supply on different physiological parameters were investigated in fenugreek. Seedlings were grown in plastic pots filled with inert sand under five $ZnSO_4$ treatments (C: control :1.5 µM Zn; 1 mM, 2 mM, 3 mM and 4 mM $ZnSO_4$). Results showed a decrease of 56% to 75% in shoot dry weight and a decrease of 65% to 90% in roots dry weight, relatively to the control. In addition we showed a significant reduction in photosynthetic parameters, with the highest value of CO_2 assimilation under 1 mM Zn (3.3 µmol CO_2, $m^{-2}{\cdot}s^{-1}$) and a lower value under 4 mM Zn (0.5 µmol CO_2, $m^{-2}{\cdot}s^{-1}$). The concentration of zinc in plant shoot was around two folds the control under 1, 2 and 3 mM Zn and about four folds under the maximal concentration, 4 mM Zn. In roots, we showed a progressive increase of zinc content. Increasing zinc concentration induced a significant decrease of phosphorus concentration in shoot. Fenugreek was mainly affected by zinc excess greater than 1 mM $ZnSO_4$, however at the highest concentration, fenugreek plants exhibited different adaptation strategies.

Key words: *Trigonella foenum-graecum*, heavy metals toxicity, soil contamination, mineral nutrition, plant physiology

Introduction

Zinc (Zn) is an essential micronutrient that is needed by plants for growth and varied biochemical and physiological pathways (Dinesh et al., 2018). It plays a vital role as a constituent of metalloenzyme and a cofactor for some enzymes such as dehydrogenases, oxidases and peroxidases (Broadley et al., 2007). It is involved also in the synthesis of chlorophyll and carotenoids, auxin, nucleic acids and proteins. Furthermore, it plays an important role in photosynthesis and in the regulation of cytoplasmic concentration of nutrients, structural stability of cell membranes and proteins, as well as protection of biomembranes against oxidative damage (Cakmak, 2000; Paunov et al., 2018; Dos Santos et al., 2019). Nevertheless, high levels of this element can induce toxic effects to sensitive plants. Indeed, frequent Zn contamination of surface soils originating from prolonged use of Zn fertilizers, input from industrial pollution, and mining activities may lead to Zn toxicity in plants (Martens and Smolders, 2013).
Broadley et al., (2007) pointed out that high levels of Zn could inhibit plant growth and evolution by inducing a perturbation in the absorption and the translocation of nutrients or by interfering with metabolic processes and antioxidant defense system. Indeed, excess Zn induce generally functional disorders in plants such as plasma membrane permeability damage and photosynthesis inhibition, as well as interference with phosphorous, magnesium, and manganese uptake (Sagardoy et al., 2009; Paunov et al., 2018; Khan et al., 2019; Szopiński et al., 2019; Dobrikova et al., 2021).

* Corresponding author

Zn toxicity symptoms include stunted growth, leaf chlorosis, necrotic spots, root system damage and disturbance in water balance (Sagardoy et al., 2010). Plants differ in their ability to tolerate elevated concentrations of Zn in the soil (Rascio and Navari-Izzo, 2011; Andresson et al., 2018). Similarly, to other countries, Tunisia faces the problem of Zn toxicity, notably in soils around open-cast mining.
The culture of legumes in marginal and moderately contaminated soils could be beneficial in double ways. In fact, legumes with their specific characteristic to fix symbiotically the nitrogen in association with rhizobia can contribute to the rehabilitation and the decontamination of these soils. In the other way, moderately contaminated soils could be additional areas for the culture of some heavy metals-tolerated legumes. To verify this hypothesis, the selection of legumes species according to their Zn tolerance and metal accumulation potential was a subject of several researches. Zribi et al. (2015) showed that *Medicago sativa* under nitrogen fixing symbiosis could tolerate 2 mM Zn and accumulate Zn in their roots and promoting the phytostabilisation process. A study carried out on the behaviour of *Trifolium repens* growing in a field polluted with Cd, Pb and Zn showed that the metals were preferentially accumulated in the roots than in the aerial part and exposed the plants to the oxidative stress (Bidar et al., 2007).
Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family of *Fabaceae* and it is cultivated in many countries such as India, Pakistan, Egypt, France, Yemen, Spain, Turkey, Morocco, China and Tunisia. India is the largest producer in the world (Zandi et al., 2015). Fenugreek can be grown easily in low-input, marginal environment. It is a precious aromatic and medicinal plant used for its healthy and nutritious benefits, as it contains a considerable number of vitamins, proteins and minerals (Zandi et al., 2015; Ahmad et al., 2016). By contrast, these virtues of fenugreek cannot deny that this plant is somehow known by its considerable ability to stock enormous amounts of particular heavy metals (Pattnaik and Reddy, 2011).
The aim of this study was to investigate the behavior of fenugreek under Zn excess in term of growth, photosynthesis activity and nutrition and to verify if this legumes species is appropriate to be cultivated in Zn contaminated soils.

Materials and methods

Plant material and germination conditions

Seeds of fenugreek plant (*Trigonella foenum-graecum* L.) were disinfected and sterilized with ethanol (70%) for 1 minute, then they are rinsed several times with distilled water. The seeds were soaked in distilled water for 3 hours and germinated in darkness at 25 °C in in Petri dishes on filter paper moistened with distilled water. Three-day-old seedlings were then transferred for 52 days into plastic pots (0.5 L) filled with sterile sand (three seeds were replicated in every pot and before the start of treatments only the performant seedling was retained). The experiment was carried out in controlled greenhouse (25 °C/19 °C, 16 h photoperiod, and 60% relative humidity).

Plants were irrigated with nutrient solutions (VADEZ et al., 1996) added by different Zn concentrations. The nutrient solution contained the following concentrations of macro and micro-elements: KH_2PO_4 (0.36 mM); K_2SO_4 (0.7 mM); $MgSO_4$, $7H_2O$ (1 mM); $CaCl_2$ (1.65 mM); HBO_3 (4µM); $MnSO_4$, H_2O (6.6 µM); $ZnSO_4$, $7H_2O$ (1.55 µM); $CuSO_4$, $5H_2O$ (1.56 µM); Na_2MoO_4, $7H_2O$ (0.12 µM); $CoSO_4$, $7H_2O$ (0.12 µM) and Fe (1.26 $mg \cdot l^{-1}$). The pH of the nutrient solution was adjusted to 7. A concentration of 1.55 µM $ZnSO_4$ was used as control (C), and the excess Zn treatments were 1 mM (Zn1), 2 mM (Zn2), 3 mM (Zn3) and 4 mM $ZnSO_4$ (Zn4). The experiment was set up in a completely randomized design with five replications (5 pots per treatment) and the irrigation was performed homogeneously with 40 ml of the nutritive solution in each pot (estimated after field capacity calculation), generally two times per week.

Growth parameters

Fifty-two days old plants were harvested and separated into shoots and roots. Roots were rinsed three times with cold distilled water and blotted with filter paper. The fresh weight (FW) was immediately determined, while the dry weight (DW) was determined after drying in an oven at 60 °C until constant weight. The length of the primary root was also measured at the final harvest with a ruler.

Tolerance index (TI) was calculated as the ratio between whole plant dry weights of plants cultivated in presence of Zn and the whole plant dry weight of control plants (ULLAH et al., 2020).

Water relations

Tissue water content (TWC) was determined using the following equation:

$$TW\ (ml \cdot g^{-1}\ DW) = (FW\text{-}DW)/DW$$

Where FW is fresh weight determined 2 h after harvest, and DW is dry weight obtained after drying at 60 °C to constant weight.

Pigment content and gas exchange

Leaf chlorophyll and carotenoid concentrations ($mg \cdot g^{-1}$ FW) were determined spectrophotometrically according to ARNON (1949) and MC KINNEY (1941). Five ml of acetone 80% were added to fresh leaf samples. Chlorophyll (*a*, *b*) and carotenoid concentrations were measured at 645, 663 and 460 nm respectively according to the equations reported by MC KINNEY (1941).

CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E) and water use efficiency [WUE = A/E] were determined on fenugreek leaves just before harvest by using a portable infrared CO_2/H_2O gas exchange system (LCPro+, UK). Measurements were carried out between 10:00 and 13:00 h on the youngest fully emerged leaf (n = 3 leaf samples taken from three different plants per treatment). Data were automatically collected every minute after the photosynthesis rate had stabilised.

Ions content

Desiccated shoot and root samples were ground to a fine powder using porcelain mortar and pestle, and then ions digestion was achieved in 4/1 (v/v) $HNO_3/HClO_4$ mixture (SOMER and UNLU, 2006). Zn, magnesium (Mg) and iron (Fe) concentrations were determined by atomic absorption spectroscopy (AAS) (PERKIN ELMER Analyst 300) and phosphorus was assayed using the vanado-molybdate method (FLEURY and LECLERC, 1943).

Translocation factor (TF) was calculated as the ratio of the metal concentration in shoots to metal concentration in roots (ZHOU et al., 2013).

Proline content

Proline content was determined according to BATES et al. (1973). Samples were homogenized in 3% (w/v) sulfosalicylic acid and centrifuged for 15 min at 14000 g. The supernatants added with ninhydrin and glacial acetic acid were then incubated for 1 h in boiling water. After cooling in an ice bath, toluene was added and proline was assayed with a spectrophotometer at 520 nm. Proline content was calculated against standard proline.

Statistical analyses

Data were analysed using the statistical software XL Stat (ANOVA I). The number of repetitions was three for ions content and gas exchange parameters and five for other parameters. Significant differences between means were separated using the Duncan test (P = 0.05).

Results

Zinc effects on plant growth and water content

A significant reduction in shoot DW was observed in fenugreek plants grown in sand culture supplemented with 1, 2, 3 and 4 mM $ZnSO_4$ (56%, 68%, 73% and 75% relatives to control, respectively) (Fig. 1A). Root DW was more sensitive to Zn toxicity than shoot DW. Indeed, root biomass decreased by 65, 73, 77 and 90% in presence of 1, 2, 3 and 4 mM $ZnSO_4$ respectively as compared to control plants (Fig. 1B).

Shoot and root lengths are similarly affected by Zn supply (Fig. 1C and 1D). Indeed, these parameters significantly decreased by 29, 39, 45 and 53% in presence of 1, 2, 3 and 4 mM $ZnSO_4$ respectively as compared to control plants. Thus, it appears that biomass accumulation is more susceptible to Zn toxicity than length.

Root/shoot (R/S) DW ratio decreased by 17, 20, 32 and 68% in plants grown with 1, 2, 3 and 4 mM $ZnSO_4$ in the culture medium respectively when compared to controls. Interestingly, Zn supply has no significant effect on both shoot and root water content of fenugreek plants as compared to control plants (Fig. 2).

Leaf gas exchange and pigment concentration

Variations in main gas exchange parameters were similar to those observed for plant biomass production. Control plants displayed the highest values of net CO_2 assimilation rate (A) (Fig. 3A), stomatal

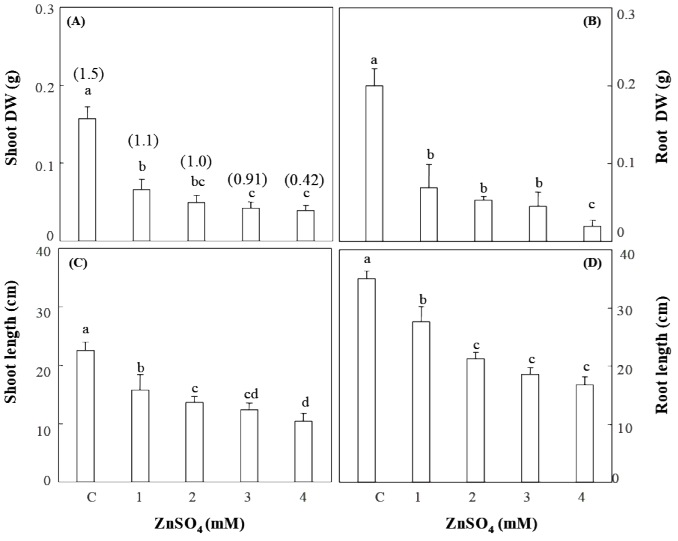

Fig. 1: Shoot (A) and root dry weight (B) and shoot (C) and root length (D) of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values on the error bars of A and B correspond to the root:shoot DW ratio, Values (means ± SE of five replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

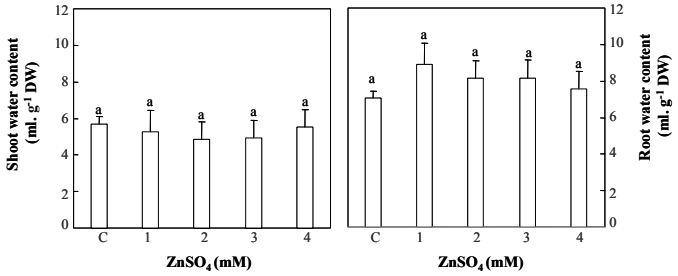

Fig. 2: Shoot and root water content of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values (means ± SE of five replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

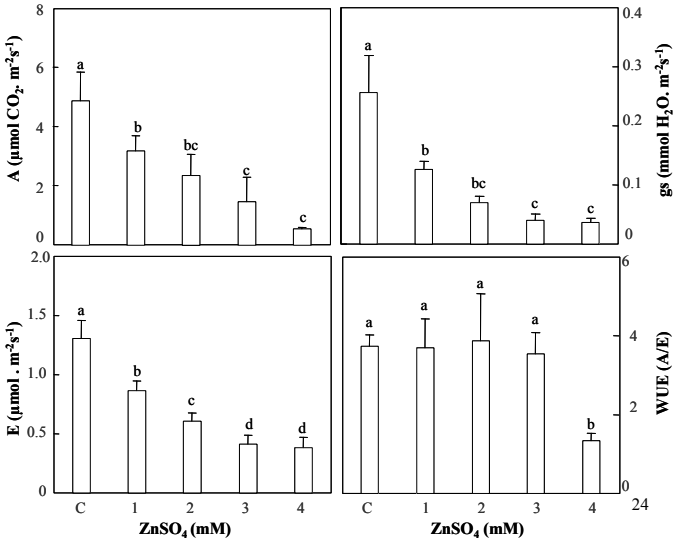

Fig. 3: CO_2 assimilation rate (A), stomatal conductance (B), transpiration rate (C) and water use efficiency (D) of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values (means ± SE of five replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

conductance (gs), (Fig. 3B) and leaf transpiration rate: E (Fig. 3C). With the increase in soil Zn content, A, gs, and E in leaves decreased significantly. CO_2 assimilation rate and transpiration rate decreased by approximately 34%, 52%, 69% and 74% in presence of 1, 2, 3 and 4 mM $ZnSO_4$ respectively as compared to control plants. Nevertheless, stomatal conductance decreased by 50, 72, 84 and 85% in Zn1, Zn2, Zn3 and Zn4 treatments respectively comparing to treated plants. It is worth mentioning that Zn supply has no significant effect on WUE in fenugreek plants (Fig. 3D).

The changes in pigments contents showed similar trends, decreasing with the increase of soil Zn content compared to control plants (Tab. 1). Chl a, b and total content decreased significantly by approximately 43, 75, 79 and 84% in presence of 1, 2, 3 and 4 mM $ZnSO_4$ respectively. Total carotenoid content decreased also by 57, 78, 85 and 89% in Zn1, Zn2, Zn3 and Zn4 plants respectively as compared to control ones. The Chl a/b ratio decreased by almost 18% in all treated plants. However, the Caro/Chl ratio decreased by 32% in presence of 1, 3 and 4 mM $ZnSO_4$ as compared to control plants.

Nutrition status

Root and shoot Zn concentrations of fenugreek plants increased generally in response to increasing Zn concentrations in the growth medium. Root Zn concentrations increased by 1.9; 2.2; 2.9 and 3.3-fold in presence of 1, 2, 3 and 4 mM $ZnSO_4$ respectively. However, shoot Zn concentrations increased by 2 -fold in Zn1 and Zn2 treatments and by 2.3 and 3.7-fold in Zn3 and Zn4 treatments respectively comparing to treated plants. It is worthy to indicate that under control conditions as well as in presence of 1, 2, and 4 mM $ZnSO_4$ the accumulation of Zn in fenugreek plants was approximately the same between roots and shoots (Fig. 4).

The rate of Zn translocation from roots to shoots was about 0.90, 0.95 and 1.0 in control and Zn1 and Zn4 treatments. It is worth mentioning that there is no significant difference in this parameter between control and Zn- treated plants.

Phosphorus (P) concentrations decreased significantly by 65% in shoots of fenugreek plants cultivated in presence of 3 and 4 mM $ZnSO_4$ but did not change significantly in roots (Fig. 5).

Tab. 1: Effect of Zn supply on leaf pigment content of fenugreek plants grown with different Zn concentrations for 52 days. Values (means SE of three replicates) followed by the same letter are not significantly different (Duncan test, P=0.05).

	C	1 mM	2 mM	3 mM	4 mM
Chla (mg.g^{-1} FW)	7.99 a	4.54 b	1.97 c	1.61 c	1.21 c
Chlb (mg.g^{-1} FW)	2.80 a	1.96 b	0.79 c	0.70 c	0.53 c
Chlt (mg.g^{-1} FW)	10.7 a	6.50 b	2.76 c	2.31 c	1.75 c
Carot (mg.g^{-1} FW)	1.62 a	0.69 b	0.35 c	0.23 c	0.17 c
Chl a/b	2.87 a	2.48 b	2.31bc	2.29 bc	2.25 c
Car/Chl	0.15 a	0.10 b	0.13 a	0.10 b	0.10 b

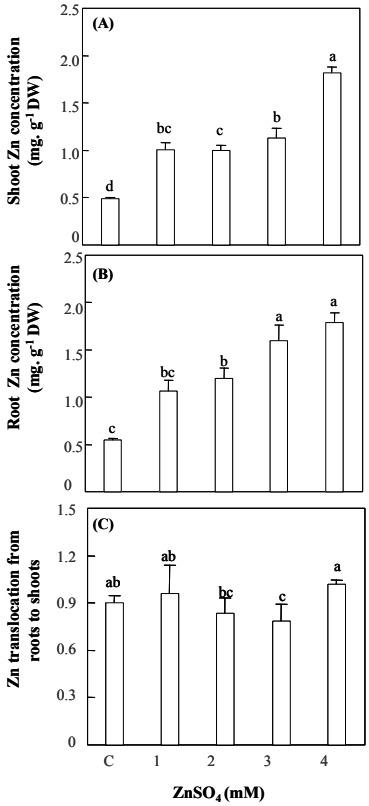

Fig. 4: Shoot Zn concentration (A), root Zn concentration (B) and Zn translocation factor (C) of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values (means ± SE of three replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

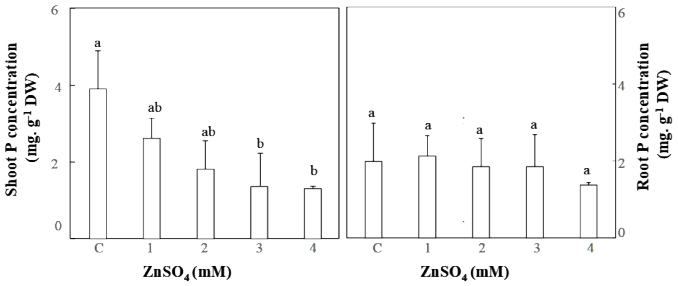

Fig. 5: Shoot and root phosphorus content of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values (means ± SE of three replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

Proline content

Our results showed that increasing Zn concentration has no significant effect on both shoot and root proline content in fenugreek plants (Fig. 6).

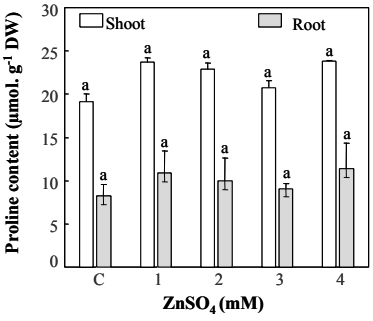

Fig. 6: Shoot and root proline content of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values (means ± SE of three replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

Discussion

The present study revealed that the increase of $ZnSO_4$ concentration in the medium leads to the reduction of fenugreek growth features. This may be due to the toxic effect of Zn that damages plant growth (Paunov et al., 2018). Indeed, lower levels of $ZnSO_4$ reduced dry weight, shoot length and root length slightly compared to higher levels (Fig. 1). Reduction of growth under excess Zn have already been described for other species and it varies in plants according to their tolerance level and experimental conditions such as concentration of the metal and the duration of the stress (Sinisha and Puthur, 2018).

Our results showed that the decrease in root biomass of fenugreek plants cultivated under Zn excess was slightly higher than in shoot biomass and R/S DW ratios decreased significantly with increasing Zn supply. These findings suggest that Zn had more inhibitory effects on the root than on the shoot. Similar results were observed by Sagardoy et al. (2009). Glińska et al. (2016) pointed out that excess Zn decreased shoot and root growth of *Triticum aestivum* seedlings likely by disturbing cell division and/or elongation. Li et al. (2013) revealed that the reduction of root growth in *Triticum aestivum* seedlings exposed to Zn excess is linked to a substantial loss of cell viability in the root tips and to an increased level of lignification. Furthermore Feigl et al. (2015) demonstrated that high Zn concentrations caused significant deposition of callose in root apical meristem of *B. juncea* and *B. napus*, which may contribute to growth inhibition because it lowers cell wall loosening and hampers symplastic transport. In the present study, fenugreek plants cultivated during 52 days in sand culture supplied with 1 mM $ZnSO_4$ showed 56% reduction in shoot DW compared to control plants. However, Al Khateeb and Al-Qwasemeh (2014) showed a 70% reduction in relative fresh weight of two solanum species: *Solanum lycopersicum* and *Solanum nigrum* grown in vitro under 1 mM $ZnSO_4$. Furthermore, the tolerance index calculated on the basis of whole plant DW of fenugreek plants cultivated in presence of 1 mM $ZnSO_4$ was 0.38 (Tab. 2) which suggest relative tolerance of this species to Zn toxicity when cultivated under 1mM $ZnSO_4$. Indeed, Lux et al. (2004) pointed out that plants with tolerance index between 0.35 and 0.6 are considered as plants with medium tolerance. However, in presence of a severe Zn stress (4 mM $ZnSO_4$), the tolerance index decreased substantially. Besides Zn vacuolar compartmentalization, cited as a potential mechanism for Zn detoxification, its retention by linking to the molecule of phytic acid in non-vacuolated tissues may also play a key role in Zn detoxification (Andressen et al., 2018).

Tab. 2: Tolerance index of fenugreek cultivated during 52 days in presence of increasing concentrations of $ZnSO_4$

	Zn1	Zn2	Zn3	Zn4
Tolerance index	0.38	0.29	0.25	0.16

Our results showed also that shoot and root length are similarly affected by Zn supply. In contrast, Feigl et al. (2016) showed no significant effect of Zn supply on root length in *Brassica napus* plants cultivated during fourteen days under 50, 150 and 300 µM $ZnSO_4$. Marichali et al., (2016) reported that the repression of root elongation of *Nigella sativa* L. exposed to Zn excess was explained by the inhibition of cell proliferation and subsequent elongation. Kaur and Garg (2021) pointed out that the decrease in growth under Zn excess is a non-specifc manifestation of alterations in physio-biochemical traits which can result from direct effects (toxicity due to accumulation in tissues) and/or from indirect effects. Indirect factors include disturbances in photosynthetic activity, limitation of minerals and water acquisition as well as the induction of oxidative stress through overproduction of ROS. Zhang et al. (2020) reported that excess Zn damages the organization of mitochondria and leads to decrease in nicotinamide levels, and consequently reduced the energy metabolism, which may explain for the diminution in overall plant growth.

Remarkably, we conclude in this research that increasing $ZnSO_4$ concentration has no significant effect in both shoot and root water content. These results suggest that osmotic adjustment in fenugreek subjected to Zn excess is efficient and that this cultivar may regulate cell osmotic potential to reduce toxic effects of Zn.

Contrarily, many studies showed a decrease in both, shoot and root water content of many species under Zn excess exposure (Sagardoy et al., 2009; Rucinska-Sobkowiak, 2016). By maintaining the water content, fenugreek seems capable to avoid the reduction in root and shoot hydraulic conductivity and the reduction in aquaporin activity, which are described as principal causes of water content decrease (Kaur and Garg, 2021). These results suggest also that fenugreek may regulate cell osmotic potential to maintain stable water status under Zn stress exposure.

The reduction of growth by Zn toxicity in fenugreek plants may be the consequence of the decrease in photosynthesis. Indeed, it was show that the plant growth is closely, related to the quantity of assimilated CO_2. Similarly, it has been shown that photosynthesis activity depend to Zn supply (Sagardoy et al., 2009 and 2010). Indeed, according to Van Assche and Clijsters (1986), high Zn concentrations can affect Rubisco activity. The decrease in photosynthesis under Zn excess exposure could be due to many factors such as the decrease in chlorophyll biosynthesis, the inhibition of the activities

of key enzymes of the Calvin cycle, the reduction in chlorophyll *a* fluorescence and the inhibition of photosynthetic electron transport (YANG et al., 2020).

Our data showed also that stomatal conductance and transpiration rate decreased significantly with increasing Zn supply. This may be interpreted as a water saving mechanism, which correlates with changes in shoot water content. Furthermore, the decrease in *gs* may be ascribed to stomatal closure or decrease of the stomatal aperture size. It was also demonstrated that the decrease in stomatal conductance under Zn excess may be related to an alternation in the K^+/Ca^{2+} ratio in the guard cells and/or to the abscisic acid concentration, which controls the stomatal movement (MARSCHNER, 1995). Another factor that is important to consider in plants under Zn toxicity is water use efficiency (WUE). We showed in this experiment that increasing Zn concentrations in the culture medium up to 3 mM $ZnSO_4$ has no significant effect in WUE in fenugreek plants.

Leaf chlorophyll content is an important physiological index directly related to photosynthesis in plants. Chlorophylls and carotenoids are involved primarily in light harvesting and a balance in their amounts is imperious for optimum light energy capture in photosynthesis (WAHID and GHAZANFAR, 2006; POLIVKA and FRANK, 2010). In the present experiment, we noted a gradual decrease in the concentrations of chlorophyll *a*, *b* total and carotenoids with the increase in $ZnSO_4$ concentrations in the soil. Furthermore, PAUNOV et al. (2018) reported a 55% decrease in Chl a content and 24% reduce in chlorophyll b content in leaves of durum wheat after 7 days treatment with only 600 µM Zn. Nevertheless, ZHANG et al. (2020) showed no significant effect of Zn stress in the content of Chl and Car in tobacco leaves cultivated during 10 days in presence of only 200 µM Zn. The decline in chlorophyll content in the plants exposed to Zn toxicity is believed to be probably due to (i) inhibition of important enzymes, such as 6-aminolevulinic acid dehydratase (ALA-dehydratase) (PADMAJA et al., 1990) and protochlorophyllide reductase (VAN ASSCHE and CLIJSTERS, 1990) associated with chlorophyll biosynthesis; and/or (ii) impairment of the supply of Mg^{2+} and Fe^{2+} (MARSCHNER, 1995). However, in this experiment, we showed that shoot Mg and iron concentration of fenugreek plants was not significantly affected by Zn supply (Fig. 7) which suggest that the decrease in chlorophyll content of fenugreek leaves was mainly due to the decrease of activities of enzymes related to chlorophyll biosynthesis. Furthermore, many studies suggest that heavy metal ions could interfere with Chl biosynthesis through central Mg ion substitution, which therefore impairs the functioning of Light Harvesting Complex II: LHCII (CENKCI et al., 2010). In this study, fenugreek plants cultivated under $ZnSO_4$ showed a significant decrease in Chl *a*/*b* ratio as compared to control plants, suggesting that Chl *a* was degraded at a higher rate than Chl *b*. Similar findings are observed in *Phasoelus vulgaris* plants cultivated under Zn excess (VASSILEV et al., 2011). Reduction of the chlorophyll *a*/*b* ratio indicate that PSI is degraded faster than PSII (ANDERSSON et al., 2004) or that LHCII remains intact longer than the reaction centres (MOY et al., 2015).

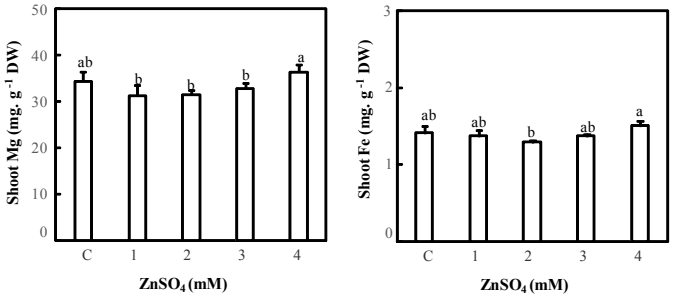

Fig. 7: Shoot content of Mg and Fe of fenugreek plants grown with different $ZnSO_4$ concentrations for 52 days. Values (means ± SE of three replicates) followed by the same letter are not significantly different (Duncan test, P = 0.05).

Similar to chlorophyll, carotenoids content also significantly decreased in response to increasing soil Zn concentrations. GIANNAKOULA et al., (2021) reported that the reduction in carotenoid content observed in many plant species during metal toxicity may be due to a protective mechanism that preserves chlorophyll pools at the expense of carotenoids, due to overproduction of reactive oxygen species.

BROADLEY et al. (2007) reported that high levels of Zn could decrease plant growth via the induction of a perturbation of the absorption and the repartition of nutrients or by interfering with metabolic processes and antioxidant defence system. In the present study, control plants showed statistically significantly lower amounts of Zn in both shoots and roots compared to all Zn-treated plants. Furthermore, the strong positive correlation between Zn in plant roots and Zn in the culture medium indicates that the Zn content in roots strongly depend on its concentration in the soil. MARSCHNER (1995) reported that relatively to the highly mobile elements such as K or P and the immobile element Ca, Zn has an intermediate mobility.

Our results showed that under control conditions as well as in presence of $ZnSO_4$, the accumulation of Zn in fenugreek plants was approximately the same between roots and shoots. RASCIO and NAVARI-IZZO (2011) pointed out that Zn repartition and translocation in plants is influenced by the level of Zn amount and plant species. At high exogenous quantities of Zn, the tolerance of plants is expressed by an accumulation of this metal in the root and the leaves. PEARSON and RENGEL (1995) have suggested that the transpiration stream may be a driving force in translocation of Zn and its accumulation in leaves. Furthermore, fenugreek plants cultivated in presence of $ZnSO_4$ accumulate high shoot Zn concentrations. It seems that fenugreek is likely characterized by an efficient Zn transport from roots to shoots. Indeed, plants tolerant to Zn toxicity can reduce the metal damage and grow optimally under Zn excess (MATEOS-NARANJO et al., 2014). However, in the present study, we found that plant biomass production was more closely related to CO_2 assimilation rate, stomatal conductance and pigment contents than to translocation factor. Indeed, a weak correlation was found between translocation factor and whole plant DW (data not shown). Thus, our findings suggest that the increasing accumulation of Zn in plant leaves subjected mainly to 4 mM $ZnSO_4$ may be attributed to the inability of fenugreek plant to chelate Zn with organic and inorganic acids to make it insoluble and limit its transport from roots to leaves. Accumulated Zn may affect the structure of the thylakoid membranes in chloroplasts and lead to a decrease of electron transport rates. Therefore, the effects of Zn stress on plant growth and biomass production were likely related to the accumulation of Zn in leaves at first and then reduced photosynthesis. HUANG et al. (2019) observed similar results in *Zelkova schneideriana* plants cultivated during 15 days in presence of increasing concentrations of Zn.

Several research accomplished in different plant types showed the presence of a reversed relationship between phosphorus (P) and Zn accumulation in plants (KHAN et al., 2019). Furthermore, Zn excess is known to interfere with Fe, P, Mg and Mn uptake by competing with these ions for binding at numerous sites, such as principal absorption region or loading region of roots (TEWARI et al., 2008). BAZIHIZINA et al. (2014) pointed out that changes in nutrient contents under Zn excess exposure may also be due to alteration in the functioning of membrane transporters and ion channels and to membrane depolarization.

In this experiment, shoot Fe content of fenugreek plants was not affected by Zn excess exposure. Similarly, YANG et al. (2011) reported that *Vitis vinifera* leaves retained high level of Fe under Zn stress, which was attributed to enhanced translocation of this element from the root to shoots.

In our present work, we found that increasing Zn amount has no

substantial consequence on root P concentrations. However, shoot P concentrations decreased significantly under Zn excess. Sagardoy et al. (2009) showed that increasing Zn supply up to 300 µM Zn increased significantly leaf P content and it has no significant effect on root P content of sugar beet plants.
Proline prevents membrane damage and had a protective role in lipid peroxidation induced by metals (Thounaojam et al., 2012). In the present study, we showed that both shoot and root proline content in fenugreek plants did not increase significantly as Zn levels increased. Contrarily, Al Khateeb and Al-Qwasemeh (2014) showed a significant increase in proline content in both *Solanum lycopersicum* and *Solanum nigrum* grown under different levels of $CuSO_4$, $ZnSO_4$ and $CdCl_2$. Parlak and Yilmaz (2012) reported also that proline content increased under Zn toxicity in three tested plants.

Conclusion

In conclusion, excess Zn in fenugreek plants caused an array of effects related to the Zn levels in the culture medium. Lower levels of $ZnSO_4$ reduced growth and physiological parameters slightly compared to higher levels. These effects are related to the accumulation of Zn in leaves and roots which, damage chloroplast functioning and caused a progressive decrease in photosynthetic rate, transpiration rate, leaf chlorophylls and carotenoids concentrations, shoot phosphorus (P) concentrations and therefore plant growth. Even at the highest Zn concentration, fenugreek plants exhibited different adaptation strategies such as closing stomata and maintain of stable water content in both shoots and roots and stable root P concentration as compared to control plants.

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Conflict of interest
No potential conflict of interest was reported by the authors.

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ORCID
Fadwa Melki https://orcid.org/0000-0002-1004-3691
Ons Talbi Zribi https://orcid.org/0000-0003-4902-6925
Sabrine Jeder https://orcid.org/0000-0001-6101-3292
Issam Nouairi https://orcid.org/0000-0003-1850-1164
Haythem Mhadhbi https://orcid.org/0000-0003-0786-4269
Kais Zribi https://orcid.org/0000-0001-8856-9573

Address of the corresponding author:
Dr. Kais Zribi, Laboratory of Legumes and Sustainable Agrosystems, Centre of Biotechnology of Borj-Cedria, BP 901, 2050 Hammam-Lif, Tunisia.
E-mail: kais.zribi@cbbc.rnrt.tn