IMPACT OF FOLIAR APPLICATION OF A-TOCOPHEROL ON GROWTH PARAMETERS, DNA INTEGRITY LOSS, CELL PROLIFERATION INCREASE, NUCLEAR MORPHOLOGY AND CHROMOSOMAL ALTERATIONS OF FABA BEAN CULTIVARS UNDER CADMIUM STRESS

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Abstract. In the present study, we attempted to clarify the mechanism that might be involved in the ameliorative effects of α-tocopherol on faba bean plants grown under different Cd levels. The effect of α-tocopherol on growth parameters, photosynthetic pigments, micronucleus formation, DNA integrity loss, cell proliferation increase, nuclear morphology and chromosomal alterations. These were studied in plants grown in a nutrient solution supplemented with different concentrations of Cd (0 or 150 μmol/l) and sprayed with α-tocopherol at concentrations of 0, 50 and 100 mg/l. Heavy metal stress caused an increase in chlorophyll a and b. Cytogenetic analysis showed that Cd could cause significant micronucleus formation and the appearance of chromosomal abnormalities affecting the structure and number of chromosomes in the root meristems of the bean. The genotoxic effect of Cd is caused by the production of ROS. It should be noted that the combination of tocopherol and Cd reduced the toxic effect of this stress at cellular level. Application of α-tocopherol helping to reduce oxidative damage and develop tolerance to heavy metal stress in α-tocopherol-treated bean plants. This α-tocopherol-induced increase in tolerance to heavy metal stress was evidenced by improvements in photosynthetic activity. The results show that treatment with α-tocopherol reduced the adverse effects of Cd stress on bean plants.
Keywords: *cadmium, Vicia faba, vitamin E, DNA damage, micronucleus, chromosomic aberration*

Introduction

Pollution of the environment and soil has become a worrying problem. The number of pollutants present in the soil is extremely high. Among the most widespread are hydrocarbons, pesticides, plastics, asbestos and heavy metals (Adriano, 2001; Alkorta et al., 2004; Bussian et al., 2021).

Heavy metals are one of the main inorganic pollutants in soil. They are continually added to the soil by various activities. Some trace elements, such as zinc (Zn), copper

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(Cu), iron (Fe) and manganese (Mn), are essential for the body, but increasing their concentration makes them toxic and dangerous for living organisms. Other elements are not necessary and can only have toxic effects. Various elements fall into this category, most notably cadmium (Cd) (Vamerali et al., 2010; El Rasafi et al., 2022).

Cadmium is one of the heavy metals that cannot be degraded in the soil, so it persists in the environment for long periods. Plants are directly exposed to this element, which has an undeniably toxic potential and is passed on to humans through the food chain (Xu et al., 2013).

The primary response of plants to heavy metal toxicity is the induction of oxidative stress through the generation of reactive oxygen species (ROS) and radicals. These species react with essential plant molecules, such as DNA, proteins and lipids. They lead to the peroxidation of membrane lipids, resulting in cellular damage and enzyme dysfunction (Ishtiyaq et al., 2018; Małkowski et al., 2019).

To deal with the damage caused by ROS, plants have developed an antioxidant defense mechanism that is made up of both enzymatic systems (catalase, superoxydedismutase, peroxidase, glutathione reductase, glutathione S-transferase, glutathione peroxidase, etc.), as well as non-enzymatic systems (vitamins C and E, carticonoids, glutathione, etc.). This latter system maintains a high antioxidant capacity in the cells and increases tolerance to stress (Shahid et al., 2014). As vitamin E is fat-soluble, it binds to membranes and can therefore sequester free radicals, preventing the propagation of lipid peroxidation reactions (Evans, 2007; Kumar and Pathak, 2018). Vitamin E (α-tocopherol) can become one of the solutions in agriculture in the fight against oxidative stress (Fritsche et al., 2017). Furthermore, there are missing gaps in understanding the proper physiological mechanism for the induction of stress tolerance at different growth stages by the exogenous use of organic compounds like that of α-Toc, also considering its translocation to specific plant parts. Therefore, the current work was aimed to quantify to which extent the foliar applied α-Tocopherol could modulate growth in water-stressed maize plants and when it should be applied in the early growth stage (Ali et al., 2020).

In our present work, with the aim of gaining a better understanding of the response of *Vicia faba* bean seedlings to stress caused by the presence of cadmium, we are going to study the protective effect of vitamin E against this stress.

Materials and methods

Plant material and growing conditions

The plant material used in this work is the faba bean (*Vicia faba*), variety; Aguadulce, chosen because of its use in the laboratory as a model plant for toxicology studies of various pollutants (El Hajjouji et al., 2007; Pourrut et al., 2008).

Moreover, the use of *Vicia faba* offers a number of advantages; rapid growth, high biomass and sensitivity to metals.

Seed germination

Vicia faba seeds are sorted and disinfected by washing with bleach (10%) for 20 min, then rinsed thoroughly with distilled water to remove any preservatives that have adhered to the seed. To facilitate and homogenize germination, the seeds are placed in distilled water overnight. They are then germinated between two layers of cotton soaked

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continuously in distilled water. Germination takes place in the dark at a temperature of 24 ± 2°C. After 4 days of germination, the seedlings are transplanted into buckets containing a hydroponic nutrient medium continuously aerated by aquarium pumps. The nutrient solutions are changed every 2 days to avoid pH variations and depletion of mineral ions. Cultivation took place in an air-conditioned room under controlled conditions; 16 h light/8 h dark, at a temperature of 25°C/20°C and a relative humidity of 65 (±5) %. The light intensity at seedling level, provided by mercury lamps, is 150 μmol m-2 s-1. The seedlings are grown on a balanced nutrient medium enriched with macro- and trace elements (Jacobson, 1951).

The Hoagland's solution consisted of 5 mM Ca $(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 50 mM H_3BO_3, 1 mM $MgSO_4$, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 0.1 mM $(NH_4)_6Mo_7O_{24}$ and 10 μM Fe EDTA pH was adjusted to pH 5.8 with HCl. The total volume of the solution was kept constant by adding deionized water to compensate for water lost through plant transpiration, sampling and evaporation. The test solutions were changed every 3 days and pH was readjusted daily.

Metal treatment and harvesting of seedlings

Vicia faba seedlings aged 7 days are treated with cadmium added to the nutrient solution in the form of $CdCl_2$, at a constant concentration of 150 μmol/l and the following day were sprayed with different concentrations of α-tocopherol (50 mg/l, 100 mg/l). The choice of cadmium and alpha-tocopherol levels can also aim to strike a balance between the induction of stress by cadmium and the ability of alpha-tocopherol to mitigate these effects. It is important that cadmium levels are high enough to see significant effects, but not so high as to cause massive plant mortality. Similarly, alpha-tocopherol levels must be chosen to be effective without being excessive.

In this study six groups were used, each group consisting of 16 plants.

The groups are:

1. Group without treatment with cadmium or vitamin E
2. Group treated with cadmium 150 μmol/l only
3. Group treated with vitamin E at 50 mg/l
4. Group treated with combined vitamin E at 50 mg/l and cadmium at 150 μmol/l
5. Group treated with vitamin E 100 mg/l
6. Group treated with combined vitamin E at 100 mg/l and cadmium at 150 μmol/l

This treatment was carried out with the nutrient solution renewed every 7 days for 2 weeks later**.**

The seedlings are harvested after 14 days of treatment. The seedlings are divided into two parts roots and leaves.

Methods

Morphological parameters

After harvesting, the whole plant is weighed and then the two parts separately using precision scales. The lengths of each part are measured using a graduated ruler to determine the number of leaves. The two parts of two planes from each batch are dried in an oven at 80°C for 24 h. They are then weighed using a precision balance.

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Extraction of chlorophyll

The chlorophyll is extracted in amixture of acetone and ethanol (75% and 25%) in volume and 80% and 20% in concentration. In fact, 100 mg of plant material cut into small pieces (the leaves are placed in black boxes to avoid oxidation of the chlorophyll by light) is added to 10 ml of a mixture of acetone and ethanol of respective volumes 75 and 25% with two concentrations of 80 and 20%. After 10 min centrifugation at 5000 rpm^{-1} at 4°C, the optical densities of the solutions were read using a spectrophotometer. Pigments contents were calculated using the equations of Lichtenthaler and Wellburn (1983).

The micronucleus test and chromosome aberration

The aim is to demonstrate the presence of an increased number of chromosomal alterations in the nuclei of *Vicia faba* root cells following exposure to cadmium. The seeds were germinated and then treated. For each treatment, 3 replicates were prepared. 2 to 3 cm of the meristematic zones were recovered and fixed in Carnoy's solution (or in a 1:3 acetoethanol solution) overnight in the dark at 4°C. The roots were preserved in 70% ethanol, then rinsed with distilled water for 10 min and hydrolysed with HCl (1N) to dissociate the cellular structures. The root cap is removed. The first 2 mm of the meristematic part are crushed on a microscope slide and stained with Feulgen stain (De Tomasi, 1936) The preparation is observed under light microscopy.

Statistical analysis

The results were expressed as mean ± SD of three different trials. We used the ANOVA one-way analysis of variance test and Tukey's test to compare the means with each other and with the controls, using R software (https://ropensci.org/blog/2021/11/16/how-to-cite-r-and-r-packages/).

Results

Morphological parameters

Bean seedlings treated with 150 µmol/l Cd showed a delay in leaf development (*Fig. 1a*) compared with the control. However, compared with the control, in the absence of cadmium, the addition of α-tocopherol at different concentrations (50 mg/l and 100 mg/l) to the nutrient solution induced an increase in leaf numbers.

Similarly, $CdCl_2$ inhibited the growth of aerial parts and significantly reduced root growth (*Fig. 1c*). Phenotypically, a browning of the root system and a reduction in the size of secondary roots were observed after treatment.

In addition, our results also showed that the interaction of α-tocopherol with cadmium has a positive effect in mitigating the harmful impact of applied metal stress on plant growth. The application of both concentrations of α-tocopherol (50 and 100 mg/l) promoted the growth of seedlings, leading to an increase in leaf development, average stem length and a significant increase in roots in the cadmium-treated batches.

Statistical analysis indicated that the difference in variations in stem elongation for all batches of *Vicia fab*a seedlings was significant (P = 0.0491), while root elongation was highly significant (P = 0.00803). However, the result indicates that there is no difference in number of leaves (p = 0.0924).

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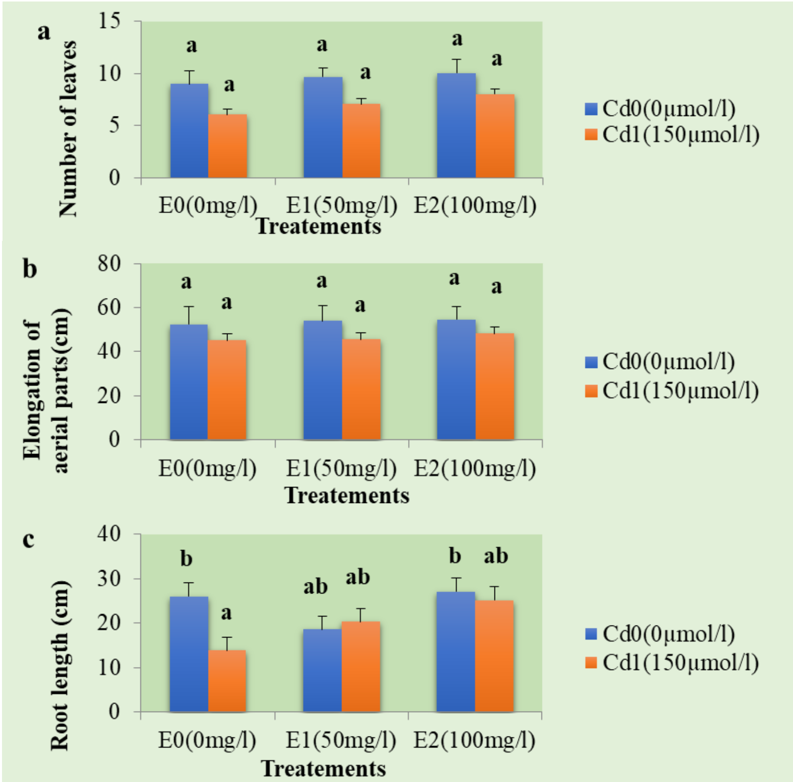
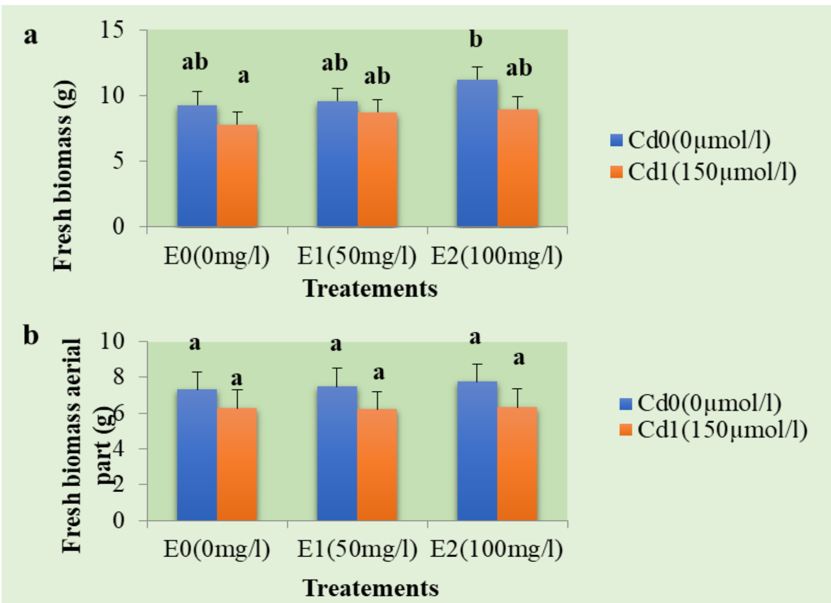


Figure 1. *Effects of α-tocopherol on leaf number (a), elongation of aerial parts (b) and root elongation (c) in bean seedlings subjected or not to cadmium stress*

Fresh biomass

Bean seedlings treated with 150 µmol/l Cd did not show a decrease in stem fresh biomass until the end of treatment (*Fig. 2a*), whereas the reduction in root fresh matter (*Fig. 2b*) was significant compared to whole plant fresh biomass at a concentration of 150 µmol/l Cd.



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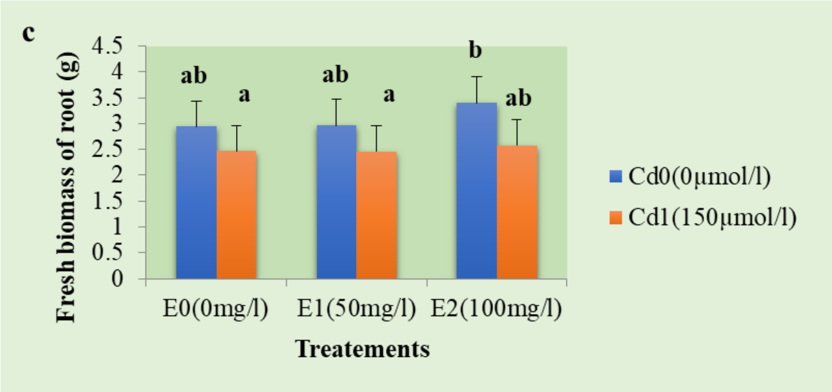

Figure 2. *Effects of α-tocopherol on plant fresh matter (a), aerial part (b) and root part (c) in bean seedlings with and without cadmium stress*

However, compared with the control, in the absence of cadmium, the addition of α-tocopherol to the nutrient solution induced a significant increase in fresh plant biomass, stems and a significant increase in root weight compared with the stressed group.

Our results also showed that the interaction of α-tocopherol with cadmium had a positive effect on the fresh biomass of stressed plants. The application of the two concentrations of α-tocopherol (50 and 100 mg/l), led to an increase in the fresh biomass of the plants, stems and roots of the batches treated with cadmium. Statistical analysis indicates that there is no difference in stem fresh biomass variation ($P = 0.0601$) while plant and root fresh biomass is highly significant ($P = 0.0287$, $P = 0.0105$).

Dry biomass and moisture content

Our results show that bean plants treated with 150 µmol/l Cd show a reduction in above-ground dry biomass only at the end of the treatment (*Fig. 3a*), while the reduction in root dry matter (*Fig. 3b*) is greater than in the control.

However, compared with the control, in the absence of cadmium, the addition of α-tocopherol to the nutrient solution resulted in an increase in stem and root dry biomass. Our results also show that the interaction of α-tocopherol with cadmium has a positive effect on the dry biomass of stressed plants. The application of the two concentrations of α-tocopherol (50 and 100 mg/l) led to an increase in the dry biomass of the stems and roots of the cadmium-treated batches. Statistical analysis indicated that there was no difference in stem ($P = 0.508$) and root ($P = 0.834$) dry biomass variations.

Figure 3 illustrates the effect of α-tocopherol at different concentrations on water content in the two parts of the bean subjected or not to cadmium stress.

In the aerial part (*Fig. 3c*), we found that the water content showed a significant difference between the groups ($P = 0.0208$), for a treatment of 150 µmol/lde $CdCl_2$, shows an increased dehydration of the aerial parts in response to the presence of cadmium in the culture medium, while exogenous application of α-tocopherol at 100 mg/l only shows a significant increase in the water content of the bean compared with the group stressed by Cd alone.

In contrast to the above-ground part, root water content (*Fig. 3d*) was less affected by cadmium, which did not differ significantly between groups ($P = 0.238$).

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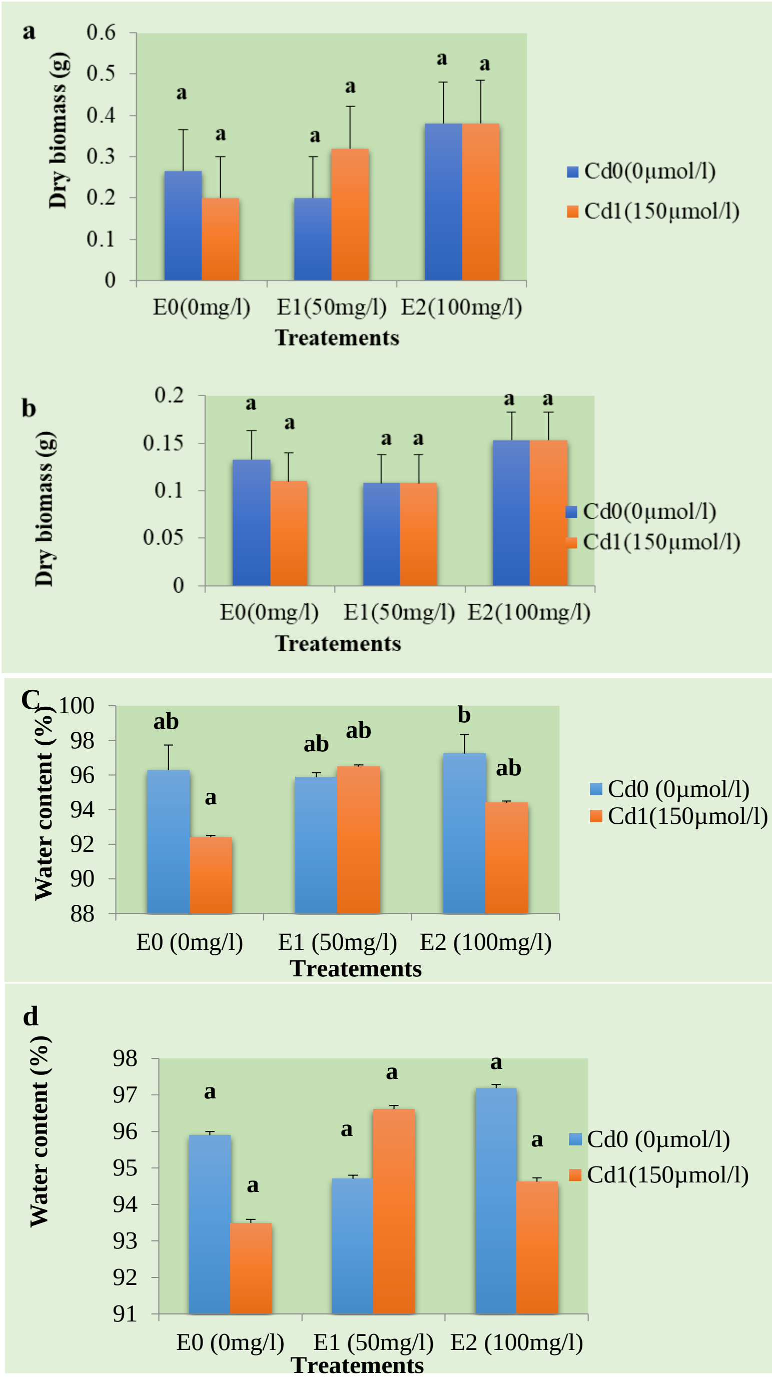

Figure 3. *Effects of α-tocopherol on dry matter of the aerial part (a) and root part (b), water content of the aerial part (c) the water content and root part (d) in bean seedlings subjected or not to cadmium stress*

Photoreceptor pigments

According to our results, we noted a significant reduction in chlorophyll b ($p = 0.0102$) (*Fig. 4b*) following treatment with 150 µmol/l Cd, while an insensitivity of chlorophyll a (*Fig. 4a*) and chlorophyll a + b (*Fig. 4c*) was observed. We also noted that the application of both concentrations of α-tocopherol to the seedlings resulted in an

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increase in Chlo, b and a + b compared with the control and specifically a significant increase in chlo b compared with the stressed group ($p = 0.0102$).

In addition, our results also showed that the interaction of α-tocopherol with cadmium has a positive effect in mitigating the harmful impact of metal stress applied to bean seedlings. Application of the two concentrations of α-tocopherol (50 and 100 mg/l) led to an increase in chlorophyll a,b and a + b. Statistical analysis indicated that the difference in chlorophyll b variations between all batches of *Vicia faba* seedlings was significant ($P = 0.0102$), whereas there was no significant difference between batches in chlorophylls a and a + b ($P = 0.372$, $P = 0.0819$).

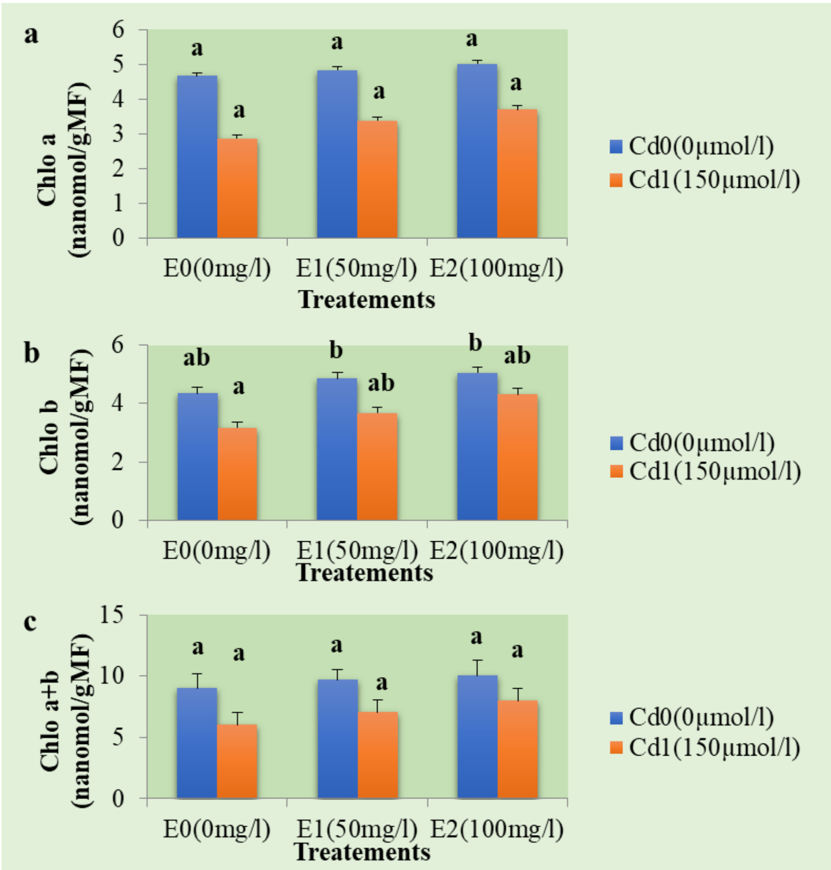

Figure 4. *Effects of α-tocopherol on chlorophyll a (a), chlorophyll b (b) and chlorophyll a + b (c) content in bean seedlings subjected or not to cadmium stress*

The formation of micronuclei

Figure 5 shows our results concerning the induction of micronuclei/1000 cells based on the microscopic observation of *Vicia faba* root tips under the combined effect of Cd and α-tocopherol at different concentrations during 24, 48 and 72 h of treatment.

The presence of Cd 150 µmol/l induced an increase in micronucleus formation after 24 h of exposure (*Fig. 5a*). Micronucleus counting showed that micronucleus production began significantly ($p = 0.0185$), while the combination of stress and α-tocopherol (50 mg/l and 100 mg/l) led to a reduction in micronucleus formation as the concentration of this vitamin increased, whereas the control group and the groups treated with α-tocopherol alone were almost devoid of micronuclei.

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Following treatment for 48 h (*Fig. 5b*), a significant frequency of micronuclei was observed compared with 24 h. Exposure of *Vicia faba* meristematic cells to nutrient solution containing only 150 µmol/l Cd resulted in a highly significant increase in micronuclei compared with the control group.) Exogenous application of tocopherol alone was found to prevent micronucleus formation at any dose, but in the stressed groups the increase in concentration of this vitamin became significant in reducing the micronucleus rate. After 72 h of treatment (*Fig. 5c*), there was a significant increase in the formation of micronuclei compared with the preservative tests (24 h and 48 h). Statistical analysis showed a highly significant difference between the six groups (P = 0.000112), in fact, cadmium stress induced a high sensitivity to micronucleus induction and this genotoxicity was higher than in the control group, which had no micronuclei. We observed that treatment with α-tocopherol at 100 mg/l was more effective in preventing the formation of micronuclei than the 50 mg/l concentration, regardless of the group with or without cadmic stress.

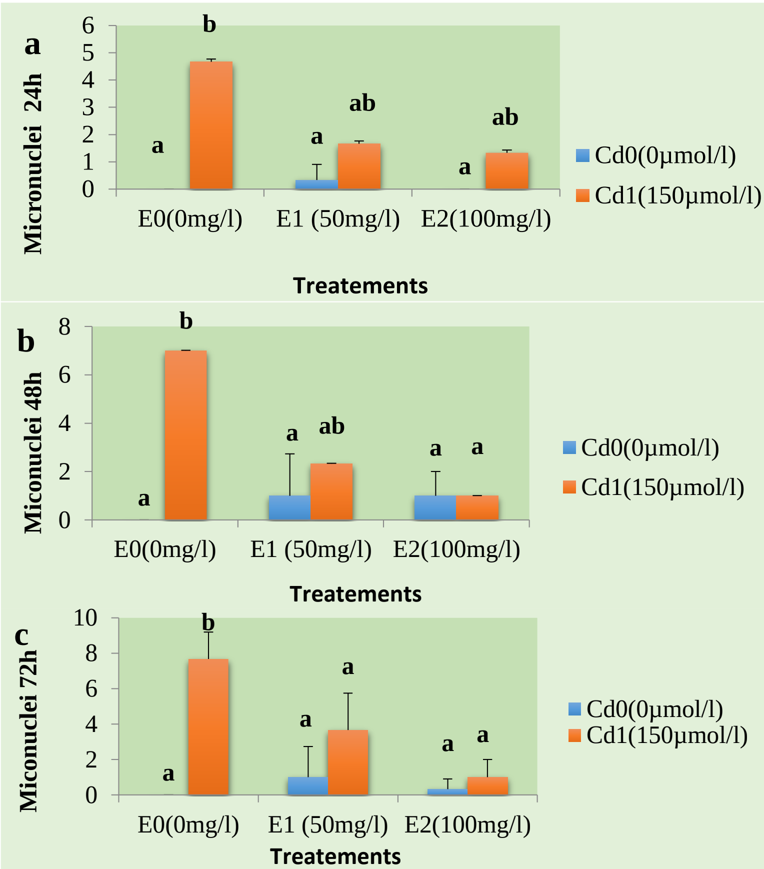

Figure 5. *Effects of α-tocopherol on the induction of micronuclei/1000 cells based on microscopic observation of root tips in bean seedlings subjected or not to cadmium stress after 24 (a), 48 (b) and 72 h (c) of treatment*

Chromosomal aberrations

During microscopic observations, in addition to the formation of micronuclei, other types of anomalies were observed which also affected the chromosomes of *Vicia fabas under* the effect of Cd 150 µmol/l.

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Our results show that the root tips of the bean seedlings divide normally and microscopic observation reveals no chromosomal abnormalities in the control group (*Fig. 6*).

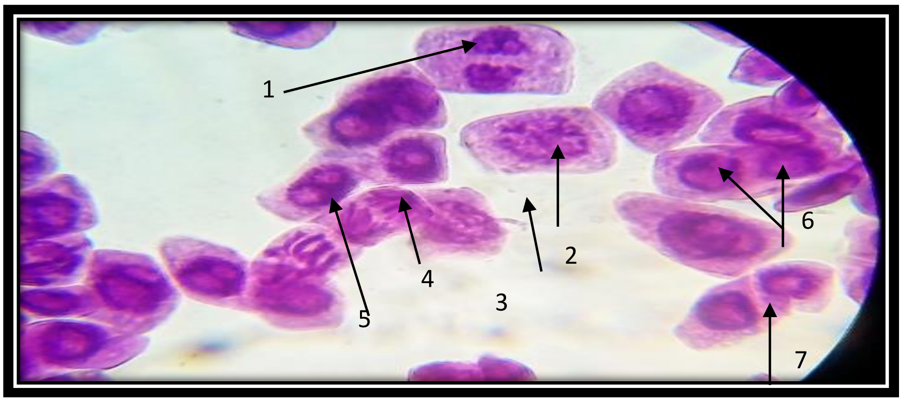

***Figure 6.** Different stages of cell division in the root meristematic zone of Vicia faba seedlings (control) 1: interphase, 2: prophase, 3: early metaphase, 4: metaphase, 5: anaphase, 6: end of anaphase, 7: telophase*

In all cases, exogenous application of α-tocopherol at different concentrations resulted in increased cell division compared with the control group, with a low frequency of chromosomal aberrations (*Fig. 7*).

***Figure 7.** Cells in the root meristematic zone of Vicia faba seedlings treated with α-tocopherol (50 mg/l and 100 mg/l) Gx40*

The figures below show that root meristematic cells treated with 150 µmol/l CdCl2 cause chromosomal abnormalities; Abnormal cell divisions; abnormal metaphases (*Fig. 8*).

Cells with structurally altered chromosomes (clastogenicity). In this case, the presence of adherent chromosomes (*Fig. 9*) and anaphase bridges (*Fig. 10*) could be detected.

Cells showing poor chromosome segregation in the form of isolated chromosomes due to non-migration of chromatids (*Fig. 11*). These chromosomes result from a delayed movement of chromatids (mismatch) during anaphase.

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Cells representing lagging chromosomes that have not joined the equatorial plate in metaphase (*Fig. 12*).

We also detected the presence of cells with micronuclei (*Fig. 13*).

Figure 8. *Abnormally dividing cells in the group stressed by Cd 150 µmol/l abnormal metaphase Gx100*

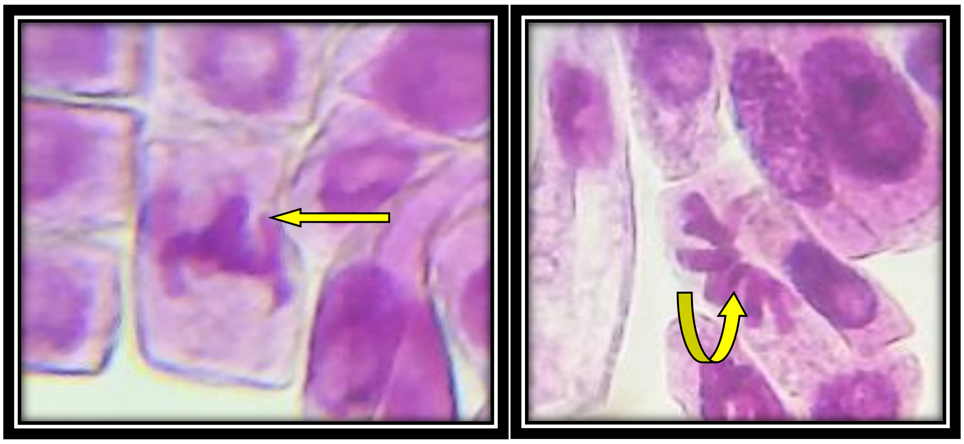

Figure 9. *Induction of adherent chromosomes in the root meristematic zone of Vicia faba in the group stressed by Cd 150 µmol/l Gx100*

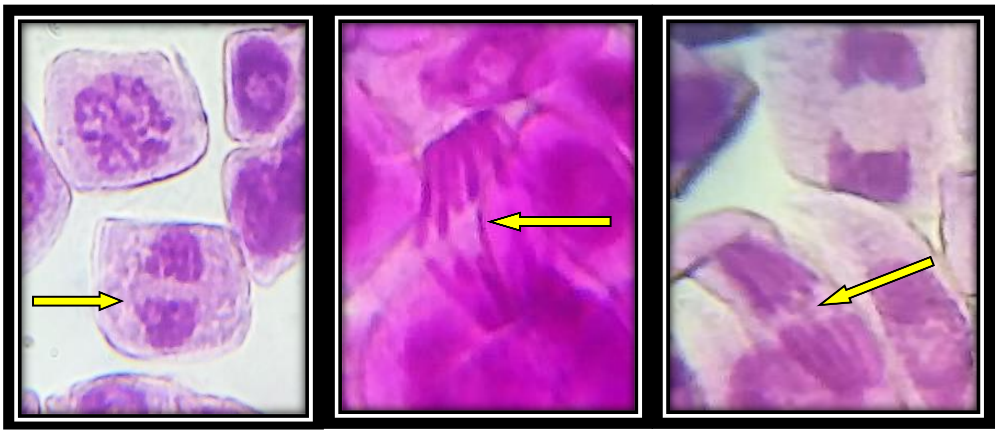

Figure 10. *Induction of several anaphase bridges in the meristematic zone of Vicia faba in the group stressed by Cd 150 µmol/l*

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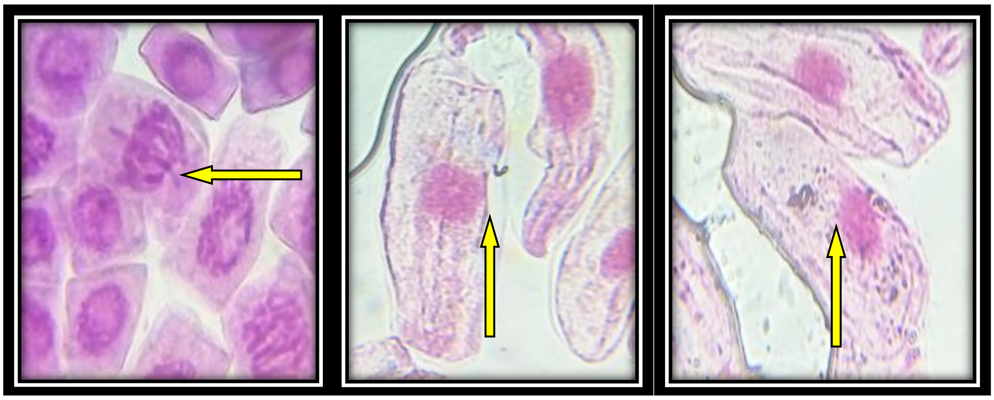

Figure 11. *Formation of isolated chromosomes in the meristematic zone of Vicia faba in the group stressed by Cd 150 µmol/l Gx100*

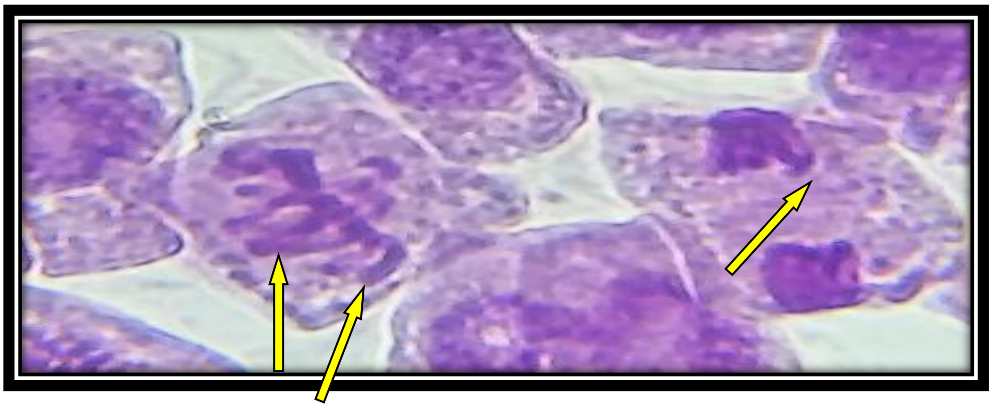

Figure 12. *Formation of non-disjoined chromosomes isolated in the meristematic zone of Vicia faba in the group stressed by Cd 150 µmol/l Gx100*

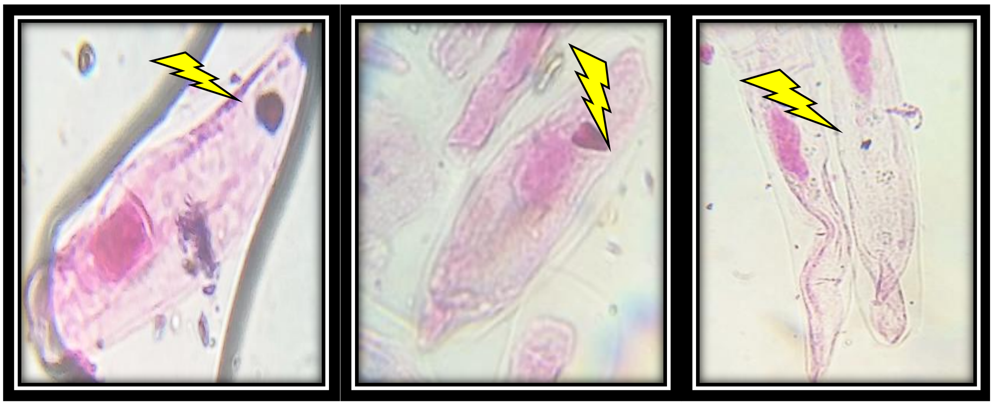

Figure 13. *Formation of micronuclei isolated from the meristematic zone of Vicia faba in the group stressed with Cd 150 µmol/l Gx100*

Discussion

Environmental or abiotic stresses, such as pollution, salinity and temperature drops, are conditions that affect plant growth and yield. Unlike animals, which can move when

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living conditions are no longer favorable, plants have developed adaptation strategies to respond to environmental changes by controlling and adjusting their metabolic systems (Noreen et al., 2018; Nawaz et al., 2019).

In particular, metal stress affected morphological aspects, certain growth parameters and photoreceptor pigment content.

In terms of the root system, we observed a browning of the roots of seedlings treated with 150 μM $CdCl_2$. This result is in line with the work of Misra et al. (2010), who also showed browning of *Bacopamonnier* roots following equivalent treatment with 100 μM Cd for 4 and 7 days. Similarly, Elloumi et al. (2014) reported browning of *Prunus dulcis* roots exposed to Cd for 14 days. This symptom of Cd toxicity at root level therefore seems to be a general phenomenon (M'rah et al., 2023; Cherif et al., 2011).

Root elongation and branching were strongly affected in fava beans under our experimental conditions. The reduction in root growth and the reduction in the number and length of lateral roots are phenotypic traits previously observed in *Pisum sativum by* Rodriguez-Serrano et al. (2006) following treatment with a concentration of 50 μM Cd for 14 days.

Our results show the sensitivity of the root system to metal stress compared with that of the leaves. This has been demonstrated by various studies (Abdel-Salam, 2018; Misra et al., 2010; Yadav et al., 2021).

We also showed that the decrease in fresh and dry matter was earlier and greater in the aerial part compared with the aerial part. However, Ben Ammar et al. (2008) showed that 50 μM Cd caused a greater drop in dry matter in the leaves (67.2%) than in the roots (55.4%) of rapeseed seedlings after 14 days of treatment. This result suggests that the morphological changes observed in the various plant organs depend on the plant species and the duration of the treatment (Nada et al., 2007; Sundararajan et al., 2022).

In general, the application of α-tocopherol significantly increased the fresh and dry weights of the above-ground and root parts. The highest weights were recorded when α-tocopherol was sprayed at a concentration of 100 mg/l. Soltani et al. (2012); Sadiq et al. (2019) reported that application of α-tocopherol increased fresh weight and improved root shoots in *Hibiscus rosa* plants.

We also observed that the water content decreases at the aerial and root level of the bean in the presence of Cd, while the study by Souguir (2009) shows that cadmium stress leads to a decrease in water content at the root level only, however, Benderradji et al. (2011) noted that salt stress leads to a significant reduction in water content in wheat. This tissue hydration could be the result of reduced growth of the bean and, consequently, a reduction in the absorption surface of the roots (Fendereski et al., 2015).

After application of α-tocopherol at different concentrations, a strong increase was noted, explained by the beneficial effect of this vitamin in maintaining membrane stability. El-Bassiouny et al. (2005) also reported that foliar spraying of α-tocopherol on faba bean plants induced an improvement in growth parameters and yield components. α-Tocopherol helps maintain the integrity of the photosynthetic membrane under oxidative stress (Munné-Bosch and Alegre, 2002a). The most important function of tocopherols is to act as a recyclable chain reaction terminator of polyunsaturated fatty acid free radicals generated by lipid oxidation. From a biosynthetic point of view, tocopherols are members of a large multifunctional family of fat-soluble compounds called prenylquinones, which also include tocotrienols, plasto-quinones and phylloquinones (vitamin K1). Tocopherols are considered important for free radical scavenging and protection against oxidative stress (Berger et al., 2003).

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Our study of the effect of Cd on the various photoreceptor pigments showed, in our case, a reduction in total chlorophyll after the end of the treatment, this reduction only affected chlorophyll b, while chlorophyll a showed no change in content compared with the control. The reduction in total chlorophyll was also observed in *Bechmerianivea, Bacopamonnieri and Pisum sativum* exposed to Cd (Sandalio et al., 2009; Sherif et al., 2010; Mishra et al., 2006; Manzoor et al., 2022).

The degradation of chlorophyll or the inhibition of its biosynthesis, which leads to a reduction in photosynthesis, is a factor that limits the induced growth of aerial parts in the presence of Cd (Bazzaz et al., 1992; Somashekaraiah et al., 1992; Dixit et al., 2001).

Fatma et al. (2009) reported that foliar application of α-tocopherol increased chla, chl b and carotenoid content compared to untreated plants. El-Bassiouny et al. (2005) reported that foliar application of α-tocopherol to faba bean plants also increased chla, b and carotenoid content.

The genotoxicity of Cd was tested in the root meristems of hydroponically-grown faba beans *(Vicia faba).* Cadmium chloride causes a high rate of micronuclei and other aspects affecting chromosome structure and number.

According to our study, treatment of bean roots with 150de Cd induced the formation of micronuclei, as reported by Souguir (2009) and several authors (Steinkellner et al., 1998; Rosa et al., 2003; Ünyayar et al., 2006; Béraud et al., 2007).

Souguir et al. (2008) showed that copper has a genotoxic effect on *vicia* faba seedlings, and that micronuclei may result from the breakage of anaphase bridges, chromosome fragments induced by clastogenicity, a chromatid or an entire chromosome. Depending on the nature of the genetic material isolated, split or altered, micronuclei of different sizes are observed.

In addition to the formation of micronuclei, microscopic observations have revealed other types of anomaly affecting the chromosomes of *Vicia faba.*

In the presence of Cd, cells with structurally altered chromosomes and cells with poor chromosome segregation were observed. In the case of structural abnormalities affecting chromosome morphology, chromosome adhesions, chromosome fragments and anaphase bridges were detected, as were chromosomes isolated as a result of chromatid non-migration resulting from delayed displacement (mismatch) during anaphase (Thapa et al., 2012). Non-disjoined chromosomes resulting from the inability of sister chromatids to disjoin during anaphase lead to migration of the 2 chromatids towards one of the poles (Pizzaia et al., 2019; Guidi et al., 2020).

The appearance of chromosomal abnormalities following cadmium treatment has been observed and interpreted by Rivetta et al. (1997) and Liu et al. (2003). Cd enters the cell through Ca2+ channels located on the plasma membrane. Ca2+ can be replaced by Cd (Rivetta et al., 1997; Clarkson and Luttge, 1989), Means and Dedman (1982) indicate that Ca-dependent calmodulin (CaM) is localized specifically at the level of the mitotic spindle and seems to be involved in the processes of chromosome regulation and function through the control of microtubule polymerization and polymerization, so the presence of cadmic ions in the nutrient solution could modify the structure of the cytoskeleton. The same applies to the function of proteins, which will lead to lesions in lipids, proteins and DNA (Holubek et al., 2020).

The accumulation of reactive oxygen species and their implications in DNA damage following cadmium stress was highlighted by Kovalchuk et al. (2001) and Rosa et al. (2003).

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Conclusion

Heavy metal pollution is a major factor in environmental degradation. Heavy metals disrupt ecosystems, damage soil and water and accumulate in plants via soil-plant transfer. These metals can be transmitted to humans and animals via the food chain.

Our study looked at the toxic effect of Cd on the morphological, biochemical, enzymatic and cytogenetic parameters of *Vicia faba* species *in the* presence and absence of exogenous treatment with α-tocopherol at different concentrations.

The study of morphological and physiological parameters showed that; The stress caused by the presence of cadmium in the nutrient solution leads to disorders in the growth of bean seedlings, with a reduction in root elongation and leaf expansion, and a reduction in fresh and dry biomass. These changes are more significant in the roots than in the leaves of the bean seedlings.

Excess Cd also led to a reduction in chlorophyll b content, disturbances in water uptake and a reduction in membrane stability. In contrast, exogenous application of tocopherol improved these parameters in both stressed and unstressed plants. Vitamin E treatment reduced cadmium toxicity. This is explained by the antioxidant power of vitamin E.

Cytogenetic analysis showed that Cd could cause significant micronucleus formation and the appearance of chromosomal abnormalities affecting the structure and number of chromosomes in the root meristems of the bean. The genotoxic effect of Cd is caused by the production of ROS. It should be noted that the combination of tocopherol and Cd reduced the toxic effect of this stress at cellular level.

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REFERENCES

[1] Abdel-Salam, M. (2018): Implications of applying nano-hydroxyapatite and nano-iron oxide on faba bean (*Vicia faba* L.*)* productivity. – Journal of Soil Sciences and Agricultural Engineering 9(11): 543-548.

[2] Adriano, D. C. (2001): Bioavailability of Trace Metals. – In: Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability, and Risks of Metals. Springer, New York, pp. 61-89.

[3] Ali, Q., Tariq Javed, M., Haider, M. Z., Habib, N., Rizwan, M., Perveen, R., Al-Misned, F. A. (2020): α-Tocopherol foliar spray and translocation mediates growth, photosynthetic pigments, nutrient uptake, and oxidative defense in maize (Zea mays L.) under drought stress. – Agronomy 10(9): 1235.

[4] Alkorta, I., Hernández-Allica, J., Becerril, J. M., Amezaga, I. 0., Albizu, I., Onaindia, M., Garbisu, C. (2004): Chelate-enhanced phytoremediation of soils polluted with heavy metals. – Reviews in Environmental Science and Biotechnology 3: 55-70.

[5] Bazzaz, F. A., Fajer, E. D. (1992): Plant life in a CO 2-rich world. – Scientific American 266(1): 68-77.

[6] Ben Ammar, W., Mediouni, C., Tray, B., Ghorbel, M. H., Jemal, F. (2008): Glutathione and phytochelatin contents in tomato plants exposed to cadmium. – Biologia Plantarum 52: 314-320.

[7] Benderradji, L., Brini, F., Amar, S. B., Kellou, K., Azaza, J., Masmoudi, K., Hanin, M. (2011): Sodium transport in the seedlings of two bread wheat (*Triticum aestivum* L.*)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_47434761

genotypes showing contrasting salt stress tolerance. – Australian Journal of Crop Science 5(3): 233-241.

[8] Béraud, E., Cotelle, S., Leroy, P., Férard, J. F. (2007): Genotoxic effects and induction of phytochelatins in the presence of cadmium in *Vicia faba* roots. – Mutation Research/Genetic Toxicology and Environmental Mutagenesis 633(2): 112-116.

[9] Berger, A., Monnard, I., Dionisi, F., Gumy, D., Hayes, K. C., Lambelet, P. (2003): Cholesterol-lowering properties of amaranth flakes, crude and refined oils in hamsters. – Food Chemistry 81(1): 119-124.

[10] Bussian, B. M., Eugenio, N. R., Wilson, S. C., Ceci, A., Parelho, C., Semenov, D., Yahyaabadi, M. (2021): Main Soil Contaminants and Their Fate in the Soil Environment. – In: Global Assessment of Soil Pollution: Report. Food and Agriculture Organization of the United Nations (FAO), Rome.

[11] Cherif, J., Derbel, N., Nakkach, M., Von Bergmann, H., Jemal, F., Lakhdar, Z. B. (2010): Analysis of in vivo chlorophyll fluorescence spectra to monitor physiological state of tomato plants growing under zinc stress. – Journal of Photochemistry and Photobiology: Biology 101(3): 332-339.

[12] Cherif, J., Mediouni, C., Ammar, W. B., Jemal, F. (2011): Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solarium lycopersicum*). – Journal of Environmental Sciences 23(5): 837-844.

[13] Clarkson, D. T., Lüttge, U. (1989): Mineral nutrition: divalent cations, transport and compartmentation. – Progress in Botany 93-112.

[14] De Tomasi, J. A. (1936): Improving the technique of Feulgen stain. – Stain Tech 11:137-144.

[15] Dixit, V., Pandey, V., Shyam, R. (2001): Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum L. cv. Azad*). – Journal of Experimental Botany 52(358): 1101-1109.

[16] El Hajjouji, H., Pinelli, E., Guiresse, M., Merlina, G., Revel, J. C., Hafidi, M. (2007): Assessment of the genotoxicity of olive mill waste water (OMWW) with the *Vicia faba* micronucleus test. – Mutation Research/Genetic Toxicology and Environmental Mutagenesis 634(1-2): 25-31.

[17] El Rasafi, T., Oukarroum, A., Haddioui, A., Song, H., Kwon, E. E., Bolan, N., Rinklebe, J. (2022): Cadmium stress in plants: a critical review of the effects, mechanisms, and tolerance strategies. – Critical Reviews in Environmental Science and Technology 52(5): 675-726.

[18] El-Bassiouny, H. M. S. (2005): Physiological responses of wheat to salinity alleviation by nicotinamide and tryptophan. – Changing Environments: Responses, Adaptation and Tolerance 497-519.

[19] Elloumi, N., Zouari, M., Chaari, L., Jomni, C., Marzouk, B., Ben Abdallah, F. (2014): Effects of cadmium on lipids of almond seedlings (Prunus dulcis). – Botanical Studies 55: 1-9.

[20] Evans, J. L. (2007): Antioxidants: do they have a role in the treatment of insulin resistance. – Indian Journal of Medical Research 125(3): 355-372.

[21] Fatma, E., El-Quesni, M., El-Aziz, A., Nahed, G., Kandil, M. M. (2009): Some studies on the effect of ascorbic acid and α-tocopherol on the growth and some chemical composition of *Hibiscus rosasineses L.* at Nubaria. – Ozean J. Appl. Sci 2: 159-167.

[22] Fendereski, F., Ghorbanli, M., Sateei, A. (2015): Effects of copper heavy metal and interaction with nitric oxide on growth parameters, photosynthetic pigment, soluble carbohydrate content and antioxidant enzymes in *Portulaca oleracea.* – Iranian Journal of Plant Physiology 5(3):1411-1423.

[23] Fritsche, S., Wang, X., Jung, C. (2017): Recent advances in our understanding of tocopherol biosynthesis in plants: an overview of key genes, functions, and breeding of vitamin E improved crops. – Antioxidants 6(4): 99.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_47434761

[24] Guidi, P., Bernardeschi, M., Palumbo, M., Genovese, M., Scarcelli, V., Fiorati, A., Frenzilli, G. (2020): Suitability of a cellulose-based nanomaterial for the remediation of heavy metal contaminated freshwaters: a case-study showing the recovery of cadmium induced dna integrity loss, cell proliferation increase, nuclear morphology and chromosomal alterations on *Dreissena polymorpha*. –Nanomaterials 10(9): 1837.
[25] Holubek, R., Deckert, J., Zinicovscaia, I., Yushin, N., Vergel, K., Frontasyeva, M., Chmielowska-Bąk, J. (2020): The recovery of soybean plants after short-term cadmium stress. –Plants 9(6): 782.
[26] Ishtiyaq, S., Kumar, H., Varun, M., Kumar, B., Paul, M. S. (2018): Heavy Metal Toxicity and Antioxidative Response in Plants: An Overview. –Hasanuzzaman, M., Nahar, K., Fujita, M. (eds.) Plants under Metal and Metalloid Stress. Responses, Tolerance and Remediation. Springer, Singapore, pp. 77-106.
[27] Jacobson L (1951): Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylenediamine tetraacetate. – Plant Physiol 26: 411-413.
[28] Kovalchuk, O., Titov, V., Hohn, B., Kovalchuk, I. (2001): A sensitive transgenic plant system to detect toxic inorganic compounds in the environment. – Nature Biotechnology 19(6): 568-572.
[29] Kumar, P., Pathak, S. (2018): Short-term response of plants grown under heavy metal toxicity. – Heavy Metals 69(75722): 10-5772.
[30] Lichtenthaler, H. K., Welburn, A. R. (1983): Determinations of total carotenoids and chlorophylls "a" and "b" of leaf extracts in different solvents. – Biochem. Soc. Transact 11: 591-597.
[31] Liu, D., Jiang, W., Gao, X. (2003): Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. – Biologia Plantarum 47: 79-83.
[32] M'rah, S., Zaier, H., Boudali, G., Chaffei-Haouari, C. (2023): Cadmium effects on *Lupinus albus L* growth, phenolic compounds, and enzyme activities. – Research Square. DOI: 10.21203/rs.3.rs-2760514/v1.
[33] Małkowski, E., Sitko, K., Zieleźnik-Rusinowska, P., Gieroń, Ż., Szopiński, M. (2019): Heavy Metal Toxicity: Physiological Implications of Metal Toxicity in Plants. – In: Sablok, G. (ed.) Plant Metallomics and Functional Omic: A System-Wide Perspective. Springer, Cham, pp. 253-301.
[34] Manzoor, H., Mehwish, Bukhat, S., Rasul, S., Rehmani, M. I. A., Noreen, S., ... El Sabagh, A. (2022): Methyl jasmonate alleviated the adverse effects of cadmium stress in Pea (*Pisum sativum L.*): a nexus of photosystem II activity and dynamics of redox balance. – Frontiers in Plant Science 13: 860664.
[35] Means, A. R., Lagace, L., Guerriero, V., Chafouleas, J. G. (1982): Calmodulin as a mediator of hormone action and cell regulation. – Journal of Cellular Biochemistry 20(4): 317-330.
[36] Mishra, S., Srivastava, S., Tripathi, R. D., Govindarajan, R., Kuriakose, S. V., Prasad, M. N. V. (2006): Phytochelatin synthesis and response of antioxidants during cadmium stress in Bacopa monnieri L. – Plant Physiology and Biochemistry 44(1): 25-37.
[37] Misra, P., Toppo, D. D., Gupta, N., Chakrabarty, D., Tuli, R. (2010): Effect of antioxidants and associate changes in antioxidant enzymes in controlling browning and necrosis of proliferating shoots of elite *Jatropha curcas* L. – Biomass and Bioenergy 34(12): 1861-1869.
[38] Munné-Bosch, S., Alegre, L. (2002): The function of tocopherols and tocotrienols in plants. – Critical Reviews in Plant Sciences 21(1): 31-57.
[39] Nada, E., Ferjani, B. A., Ali, R., Bechir, B. R., Imed, M., Makki, B. (2007): Cadmium-induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. – Acta Physiologiae Plantarum 29(1): 57-62.
[40] Nawaz, F., Majeed, S., Ahmad, K. S., Hamid, A., Shabbir, R. N., Aqib, M., Ikram, R. M. (2019): Use Rodríguez-Serrano, M. A. R. Í. A., Romero-Puertas, M. C., Zabalza, A. N. A., Corpas, F. J., Gomez, M., Del Rio, L. A., Sandalio, L. M. (2006): Cadmium effect on

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_47434761

oxidative metabolism of pea (*Pisum sativum L.*) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo Plant. – Cell & Environment 29(8): 1532-1544.

[41] Noreen, S., Fatima, Z., Ahmad, S., Athar, H. U. R., Ashraf, M. (2018): Foliar Application of Micronutrients in Mitigating Abiotic Stress in Crop Plants. – In: Hasanuzzaman, M. et al. (eds.) Plant Nutrients and Abiotic Stress Tolerance. Springer, Singapore, pp. 95-117.

[42] Pizzaia, D., Nogueira, M. L., Mondin, M., Carvalho, M. E. A., Piotto, F. A., Rosario, M. F., Azevedo, R. A. (2019): Cadmium toxicity and its relationship with disturbances in the cytoskeleton, cell cycle and chromosome stability. – Ecotoxicology 28: 1046-1055.

[43] Pourrut, B., Perchet, G., Silvestre, J., Cecchi, M., Guiresse, M., Pinelli, E. (2008): Potential role of NADPH-oxidase in early steps of lead-induced oxidative burst in *Vicia faba* roots. – Journal of Plant Physiology 165(6): 571-579.

[44] Rivetta, A., Negrini, N., Lucchini, S., Cocucci, M. (1997): Mechanism of Cadmium Uptake in Maize Roots. – In: XXVII Annual Meeting of European Society for New Methods in Agricultural Research (ESNA)/joint organised with International Union of Radioecology (IUR). University of Ghent, Faculty of Agricultural and Applied Biological Sciences, Department of Applied Analytical and Physical Chemistry, pp. 40-46.

[45] Rodríguez-Serrano, M. A. R. Í. A., Romero-puertas, M. C., Zabalza, A. N. A., Corpas, F. J., Gomez, M., Del Rio, L. A., Sandalio, L. M. (2006): Cadmium effect on oxidative metabolism of pea (*Pisum sativum L.)* roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. – Plant, Cell & Environment 29(8): 1532-1544.

[46] Rosa, E. V. C., Valgas, C., Souza-Sierra, M. M., Corrĕa, A. X., Radetski, C. M. (2003): Biomass growth, micronucleus induction, and antioxidant stress enzyme responses in *Vicia faba* exposed to cadmium in solution. – Environmental Toxicology and Chemistry: An International Journal 22(3): 645-649.

[47] Sadiq, M., Akram, N. A., Ashraf, M., Al-Qurainy, F., Ahmad, P. (2019): Alpha-tocopherol-induced regulation of growth and metabolism in plants under non-stress and stress conditions. – Journal of Plant Growth Regulation 38: 1325-1340.

[48] Sandalio, L. M., Rodríguez-Serrano, M., del Río, L. A., Romero-Puertas, M. C. (2009): Reactive Oxygen Species and Signaling in Cadmium Toxicity. – In: Rio, L., Puppo, A. (eds.) Reactive Oxygen Species in Plant Signaling. Signaling and Communication in Plants. Springer, Berlin, pp. 175-189.

[49] Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., Pinelli, E. (2014): Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. – Reviews of Environmental Contamination and Toxicology 232: 1-44.

[50] Soltani, Y., Saffari, V. R., Moud, A. A. M., Mehrabani, M. (2012): Effect of foliar application of α-tocopherol and pyridoxine on vegetative growth, flowering, and some biochemical constituents of *Calendula officinalis L.* plants. – African Journal of Biotechnology 11(56): 11931-11935.

[51] Somashekaraiah, B. V., Padmaja, K., Prasad, A. R. K. (1992): Phytotoxicity of cadmium ions on germinating seedlings of mung bean (Phaseolus vulgaris): Involvement of lipid peroxides in chlorphyll degradation. – Physiologia Plantarum 85(1): 85-89.

[52] Souguir, D. (2009): Modification métaboliques, moléculaires et génotoxicité par le cadmium chez *vicia f.* – Thèse de doctorat, Université de Carthage, Tunisie.

[53] Souguir, D., Ferjani, E., Ledoigt, G., Goupil, P. (2008): Exposure of Vicia faba and *Pisum sativum* to copper-induced genotoxicity. – Protoplasma 233: 203-207.

[54] Steinkellner, H., Mun-Sik, K., Helma, C., Ecker, S., Ma, T. H., Horak, O., Knasmüller, S. (1998): Genotoxic effects of heavy metals: comparative investigation with plant bioassay. – Environmental and Molecular Mutagenesis 31(2): 183-191.

[55] Sundararajan, S., Shanmugam, R., Rajendran, V., Sivakumar, H. P., Ramalingam, S. (2022): Sodium nitroprusside and putrescine mitigate PEG-induced drought stress in seedlings of *Solanum lycopersicum*. – Journal of Soil Science and Plant Nutrition 1-14.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_47434761

[56] Thapa, G., Sadhukhan, A., Panda, S. K., Sahoo, L. (2012): Molecular mechanistic model of plant heavy metal tolerance. –Biometals 25: 489-505.
[57] Ünyayar, S., Celik, A., Çekiç, F. Ö., Gözel, A. (2006): Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. – Mutagenesis 21(1): 77-81.
[58] Vamerali, T., Bandiera, M., Mosca, G. (2010): Field crops for phytoremediation of metal-contaminated land. A review. – Environmental Chemistry Letters 8: 1-17.
[59] Xu, X., Cao, X., Zhao, L. (2013): Comparison of rice husk-and dairy manure-derived biochars for simultaneously removing heavy metals from aqueous solutions: role of mineral components in biochars. – Chemosphere 92(8): 955-961.
[60] Yadav, V., Arif, N., Kováč, J., Singh, V. P., Tripathi, D. K., Chauhan, D. K., Vaculík, M. (2021): Structural modifications of plant organs and tissues by metals and metalloids in the environment: a review. – Plant Physiology and Biochemistry 159: 100-112.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_47434761