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Study of the effect of chromium on the germination parameters of Fenugreek (*Trigonella foenum-gracium* L.) and Lens (*Lens culinaris*)

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Abstract. Soil contamination by heavy metals is a global environmental problem. This contamination affects agricultural crops in the area concerned. In the present study, chromium, which is a heavy metal, is evaluated for its diverse effects on seed germination and lateral growth of fenugreek and lens seeds. A chromium solution was prepared at increasing concentrations: 0, 0.02, 0.04, 0.06, 0.08, 0.1, and 0.2 mg L^{-1} for the addition of germinating seeds in petri dishes for ten days. After two days, the germination rate is calculated. For the following days the length of radicle, stem, and number of leaves are measured. The germination rate of fenugreek varies between 100 and 73.33% for the control and 0.02 mg L^{-1} of chromium respectively. However, the germination rate of the lens varies between 100% for the control and 90% for the 0.02 mg L^{-1}. The elongation of fenugreek radicle with chromium solutions shows a significant effect. However, there is no significant difference in the lens at the different concentrations. For the growth of the fenugreek stalk, it is noticed that the concentration 0.02 shows a length of 2.83 cm compared to their control which is 2.30 cm. Consequently, chromium at 0.02 mg L^{-1} stimulates growth, but at 0.2 mg L^{-1}, it inhibits it. For lens the length of the stems shows also a significant difference compared to their control. So the effect of chromium on germination parameters depends on their concentrations, as well as on the seed response itself. For our research the response of fenugreek compared to the lens at the same concentrations is different.

Key words: chromium, germination, fenugreek, lens, toxicity.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant of the Leguminosae family. It is one of the oldest medicinal plants from southeastern Europe and western Asia. It is cultivated in many parts of the world, but the major growing countries are India, Egypt, Morocco, China, Pakistan, Spain, Turkey, and Afghanistan (Madhava Naidu et al., 2011). The key advantages associated with fenugreek are that it can adapt to different environments and growing conditions, also its seeds contain various health beneficial compounds steroidal saponins; diosgenin, yamogenin, tigogenin, and neotigogenin (Ganghas et al., 2021).

Lens (*Lens culinaris* L.) is an important legume in the farming systems of the Mediterranean aea because it is a source of high-quality protein in human diet and animal consumption. It offers most practical means of solving protein malnutrition. The lens is characterized by its ability to enter into a symbiotic relationship with the bactrium Rhizobium leguminosarum in the fixation of atmospheric nitrogen. It helps in reducing the amount of added nitrogenous fertilizers to the plants (Ouji et al., 2015)

Chromium is a carcinogenic pollutant, widely recognized for its unprecedented hazardous effects on the living system as well as on the environment (Jabeen et al., 2016; Singh et al., 2017; Kumar et al., 2019). Chromium is heavily released into the environment as an industrial pollutant due to its extensive use in tanning, smelting, metal plating, refractory materials, battery manufacturing, pesticides, and fertilizers (Nagajyoti et al., 2010; Huang et al., 2018). The most stable and common valences of Cr are Cr (III) and Cr (VI), the latter, is known to be highly carcinogenic and mutagenic to humans and animals (Huang et al., 2018; Farid et al., 2019).

Also, higher Cr accumulation causes irreversible physiological, biochemical, anatomical and ultrastructural alterations in plants (Sikander Pal et al., 2012; Farid et al., 2017; Kumar et al., 2019; Rahan et al., 2019). The most common symptoms are restricted seed germination, root and shoot growth, chlorophyll biosynthesis, altered photosynthesis, transpiration, respiration, key enzyme activities and carbon assimilation (Mahmud et al., 2017; Zhao et al., 2019).

In this study, Fenugreek (*Trigonella foenum-gracium* L.) and Lens (*Lens culinaris*) seeds were used to evaluate the toxicity of Cr. The parameters studied are germination rate, growth, tolerance index, root toxicity index, seeds vigor index and germination index of fenugreek and lens under increasing concentrations of Cr.

MATERIALS AND METHODS

In order to evaluate the effect of chromium on the germination process, two different species are chosen. They belong to the Fabaceae family (Fenugreek (*Trigonella foenum-gracium* L.) and Lens (*Lens culinaris*)).

Plant materials

Healthy seeds (15 seeds) of uniform size were added to Petri dishes of 9 cm diameter, containing two layers of Joseph paper. Seeds were spaced by 1 cm betwen them.

Metal treatments and germination tests

The solution used is anhydrous potassium dichromate ($K_2Cr_2O_7$) dissolved in distilled water (Suthar et al., 2014).

The different concentrations used is (0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 mg L^{-1}). They were chosen considering the maximum accepted accumulation in water (0.1 mg L^{-1} for irrigation water) following the standards in Morocco (Order No 1276-01 of 10 Chaabane 1423, 17 October 2002) ('moroccan-standards-for-quality-of-water-for-irrigation' 2002).

The trial runs for 10 days, the first two days the petris dishes are put in an incubator in the dark, 25°C and 70% humidity. Afterwards, the rest of the time (8 days), the petri dishes were kept in the light 6 hours period on day, with the same conditions of 25 °C and 70% humidity. Triplicates of each treatment were studied with control using distilled water.

Germinated seeds were counted every two days. They are considered germinated when the radicle has pierced the integuments by 2 mm. (Zaghdoud et al., 2018).

Measurement methods and used equipment

In this study, several parameters were measured and which are:

- Germination rate: is expressed by the ratio of the number of germinated seeds on the total number of seeds, is determined by the formula 1 (Ameziane et al., 2020)

$$\text{TG\%} = \frac{\sum n}{\text{N}} \times 100 \qquad (1)$$

where n is the number of sprouted seeds and *N* is the number of tested seeds.

- Measurement of growth in length: the lengths of the radicles and stems for the two species studied were measured every two days with a caliper to determine the growth in length of the seedlings (expressed in cm).
- Tolerance index (TI)

The tolerance index is determined by the formula 2 of (El Rasafi et al., 2021)

TI = (Root length in treatment / Root length in control) × 100 (2)

- Root Toxicity Index (RTI):

The root toxicity index is determined by the formula 3 of (El Rasafi et al., 2021)

RTI = ((Root length in control - Root length in treatment) / Root length in treatment in control) × 100 (3)

- Seed vigor index

The root toxicity index is determined by the formula 3 of (El Rasafi et al., 2021)

SVI = ((RL + SL)× TG) / 100 (4)

*TG = germination rate; *RL = Root length; *SL = Shoot length.

- Germination index

The germination index is determined by the formula 5 of (Trautmann et Krasny 1998)

IG = (GE/GT) × (LE/LT) × 100 (5)

*GE = number of germinated seeds in the treatment; *GT = number of germinated seeds in the control; *LE = root length of germinated seeds in the treatment; *LT = root length of germinated seeds in the control.

Statistical analysis

The obtained results correspond to the average of three repetitions (three petri dish for each treatment). The study data were subjected to unidirectional variance analysis (ANOVA) and the average separations were made by the smallest difference (*LSD*) at the level of significance of ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of chromium on the germination rate of fenugreek and lens

Seed germination is a sequence of events that involves hydration of the dried seed, activation of cellular metabolism, followed by synthesis, of hydrolytic enzymes and degradation of seed macromolecules by newly synthesized and stored hydrolases. (Mabrouk et al., 2019)

For fenugreek the germination rate of the control and the 0.02 mg L^{-1} concentration is about 100%. From the concentration of 0.06 mg L^{-1} the germination rate decreases progressively until, it reaches 73% for the concentration 0.2 mg L^{-1} (Fig. 1, A).

In fact, seeds irrigated from 0.02 to 0.1 mg L^{-1} of chromium have a lower and not significant difference rate ($p > 0.05$) compared to the control, and significantly different to 0.2 mg L^{-1} (Fig. 1, A).

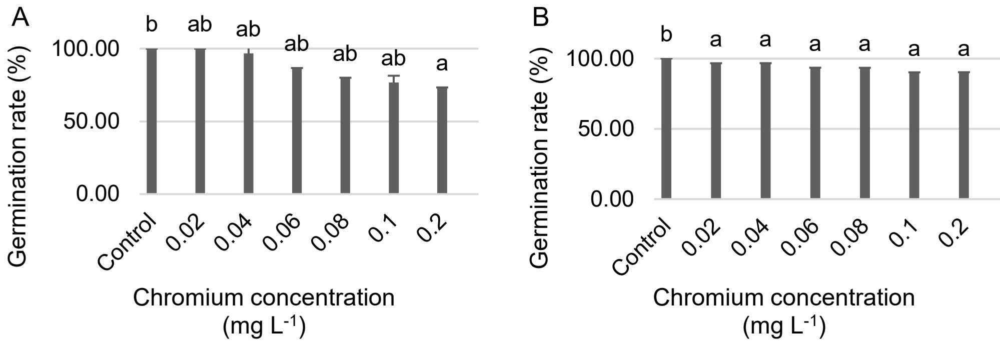

Figure 1. Effect of different chromium concentrations on the germination rate of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

For lens, the germination rate of the control is 100%. While for the concentrations 0.02 and 0.04 mg L^{-1} at a similar germination rate of about 97%. This rate decreases to a value of 90% for the concentrations 0.1 and 0.2 mg L^{-1}. The addition of chromium solution at different concentrations, on lens seeds are, negatively influenced the germination rate, but the latter remained significant compared to the control ($p < 0.05$) (Fig. 1, B).

These results are in agreement with the work of (El Rasafi et al., 2021) which shows a clear delay in germination of fenugreek seedlings exposed to As and Ni.

A similar effect has also been reported in other works, in the presence of Cr, Cu, Zn (Menon et al., 2016) Cr, Cd, Pb (Alaraidh et al., 2018) with different concentrations.

Effect of chromium on the growth of fenugreek and lens: radicles,stem and number of leaves

The treatment of fenugreek seeds with increasing concentrations of chromium, present a insignificant effect on radicle emergence (Table 1), only the concentration 0.2 mg L^{-1} has a significant effect on their control

For lens, there was a significant effect on their control. Radicles exposed to higher concentrations of chromium show a brownish coloration compared to the control (Menon et al., 2016).

These results confirm the effects found, through previous studies (El Rasafi et al., 2021; Menon et al., 2016) which demonstrated that the response of these two species under chromium stress induces a significant reduction in root growth.

The decrease in root growth in the presence of Cr^{6+} can be explained by the inhibition of root cell division and/or root cell elongation, which could have the collapse of tissues and the subsequent inability of roots to absorb water and nutrients from the environment combined with the extension of the cellular cycle (Menon et al., 2016).

The elongation of fenugreek stem is about 2.83 cm for the concentration 0.02 mg L^{-1} compared to the control which is 2.30 cm. While it dies at 0.2 mg L^{-1} in chromium (Table 1).

Table 1. Effect of different chromium concentrations on the growth of fenugreek and lens radicles,stem and number of leaves (values with different letters are significantly different ($p < 0.05$)

Treatments	Fenugreek			Lens		
	Length of radicle (cm)	Length of stem (cm)	Number of leaves	Length of radicle (cm)	Length of stem (cm)	Number of l eaves
Control	4.56 ± 0.16b	2.31 ± 0.2cd	2.72 ± 0.4c	4.07 ± 0.19b	5.57 ± 0.51b	6.08 ± 0.40c
0.02	2.45 ± 0.25 ab	2.83 ± 0.1 d	2.44 ± 0.08bc	1.66 ± 0.07a	3.25 ± 0.03ab	3.97 ± 0.42b
0.04	1.51 ± 0.21ab	2.22 ± 0.01cd	1.97 ± 0.14b	1.07 ± 0.11a	2.53 ± 0.20a	3.65 ± 0.50b
0.06	1.34 ± 0.04ab	2.05 ± 0.14c	2.17 ± 0.01bc	0.94 ± 0.22a	2.58 ± 0.52a	3.90 ± 0.57b
0.08	0.96 ± 0.01ab	1.60 ± 0.28bc	2.00 ± 0.01b	0.82 ± 0.17a	2.01 ± 0.39a	3.88 ± 0.17b
0.1	0.80 ± 0.19ab	1.05 ± 0.11b	2.00 ± 0.01b	0.98 ± 0.26a	2.24 ± 0.25a	3.67 ± 0.47b
0.2	- a	- a	- a	0.75 ± 0.35a	1.00 ± 0.01a	- a

Chromium at low concentration stimulates stem elongation and inhibits it when it increases.

Lens stem length was insignificant for the concentration 0.02 mg L^{-1}, and significantly different for concentrations ranging from 0.04 to 0.2 mg L^{-1} chromium compared to the control (Table 1).

The reduction in shoot and root length could be due to excessive salt accumulation in the cellular wall, which negatively alters metabolic activities and limits cell wall elasticity (Kanbar, 2014).

The number of leaves of fenugreek (Table 1) as a function of chromium concentration. For concentrations 0.02 to 0.06 mg L^{-1}, are not significantly different from the control. The number of fenugreek leaves is only significantly different from 0.08 to 0.2 mg L^{-1} compared to the control ($p < 0.05$), also the leaves of concentration 0.2 mg L^{-1} are dead.

The number of leaves of lens, have a significant effect ($p < 0.05$) from the concentrations 0.02 to 0.2 mg L^{-1} compared to their control. However, for the concentration 0.2 mg L^{-1}, the number of leaves are dead (Table 1).

Seed germination and root elongation have been proposed by the U.S. Environmental Protection Agency as phytotoxic indicators (ISTA, 2009). Both characters are widely and easily used as reliable phytotoxicity indicators for unstable chemical compounds, with many advantages; sensitivity, efficiency, simplicity, and relatively cheap as compared with another test (Osman et al., 2020).

Fig. 2 showed the development of radicles, stems and leaves of fenugreek and lens after 10 days. The reduction in these parameters is visually clear, growth in radicles, stems and leaves is proportional to the concentration of chromium used.

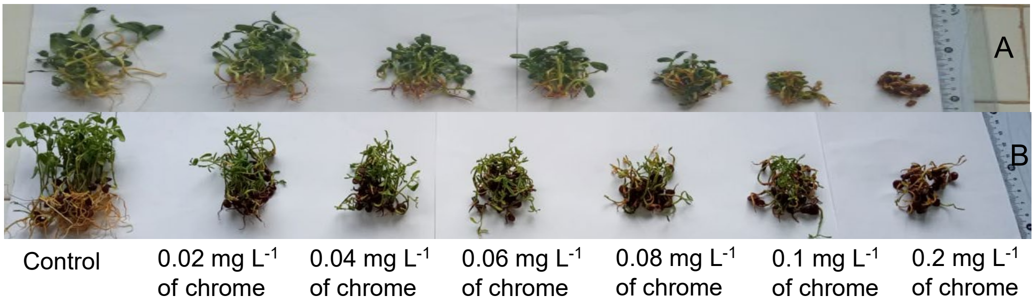

Figure 2. Development of radicles, stems and leaves of (A) fenugreek and (B) lens seedlings for different chromium concentrations.

Effect of chromium on the tolerance index of fenugreek and lens

The tolerance index (TI) was estimated for the root extension of seeds in the metal treatments and control seeds to assess the sensitivity of fenugreek and lens to different chromium concentrations (El Rasafi et al., 2021).

Fig. 3, A presents that the highest perceentage is found in fenugreek 71.76% at 0.02 mg L^{-1}. More the 0.1 mg L^{-1} the seeds of fenugreek die. The concentrations have significant effects on their control *($p < 0.05$)*.

For lens (Fig. 3, B), the tolerance index varies between 37.14% and 16.78% for the concentrations 0.02 and 0.2 mg L^{-1} respectively. The chromium concentration shows significant differences for lens ($p < 0.05$) compared to control.

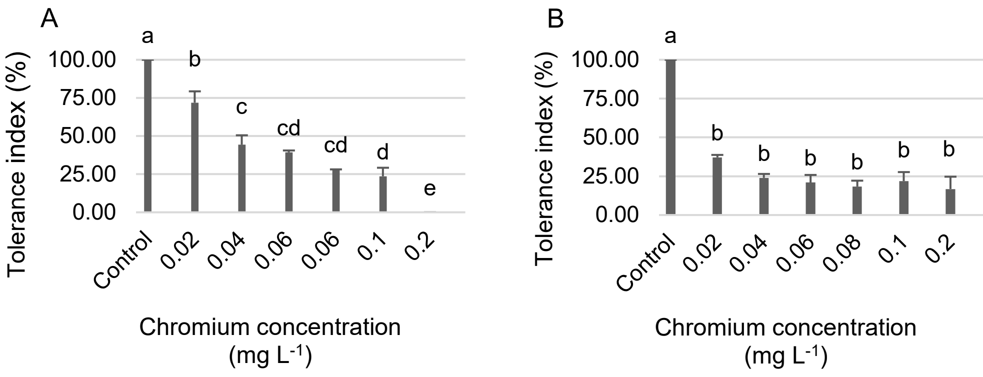

Figure 3. Effect of different chromium concentrations on the tolerance index of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

Effect of chromium on the root toxicity index of fenugreek and lens

The root toxicity index for fenugreek varies between 28.24 and 100% for the concentrations 0.02 and 0.2 mg L^{-1} respectively. For the latter, the root toxicity index has significant difference to their control ($p < 0.05$) (Fig. 4, A).

For lens, they vary from 63.70 to 89.05% for the concentrations measuring from 0.02 to 0.2 mg L^{-1} respectively. Also, a significant difference tends to diverge from the six chromium concentrations compared to their control ($p < 0.05$) (Fig. 4, B).

The toxic effect of chromium may be due to their competition for nutrient cation uptake at the root cellular level, direct interaction with functional proteins leading to disruption of their structure and function (Alaraidh et al., 2018). The inhibitory effect of chromium on amylase activities, resulting in delayed sugar transport (Katoch et Singh 2016). In addition, they interfere with cell division, leading to chromosomal aberrations and mitosis (Pavlova, 2017; Rizwan et al., 2016). Their oxidative activities and the production of free radicals (reactive oxygen species) by heavy metals in the cell can cause oxidativedamage to cells of the photosynthetic and mitochondrial apparatus (Farid et al., 2017).

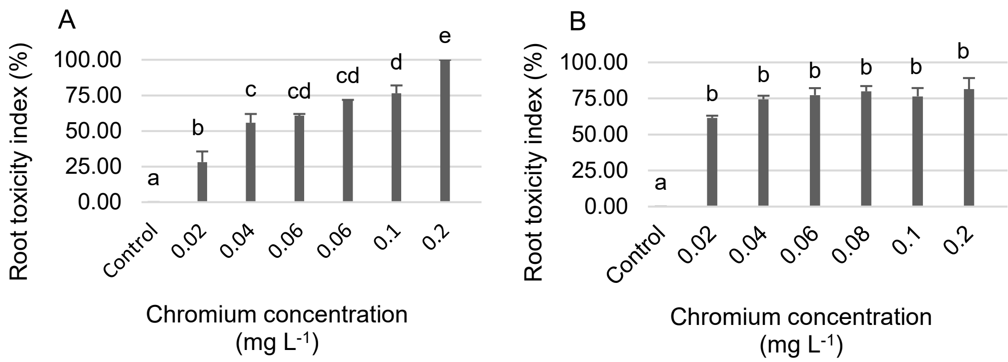

Figure 4. Effect of different chromium concentrations on the root toxicity index of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

Effect of chromium on the vigor index of fenugreek and lens

The fenugreek vigour index decreases from 528.00 to 141.50 for the concentrations 0.02 and 0.1 mg L^{-1} respectively. After that it is cancelled out for the chromium concentration of 0.2 mg L^{-1}. This is due to the fact that the fenugreek root does not resist to this concentration and dies after the sixth day with a vigour index of 29.08 against 283.76 for the control (Fig. 5, A).

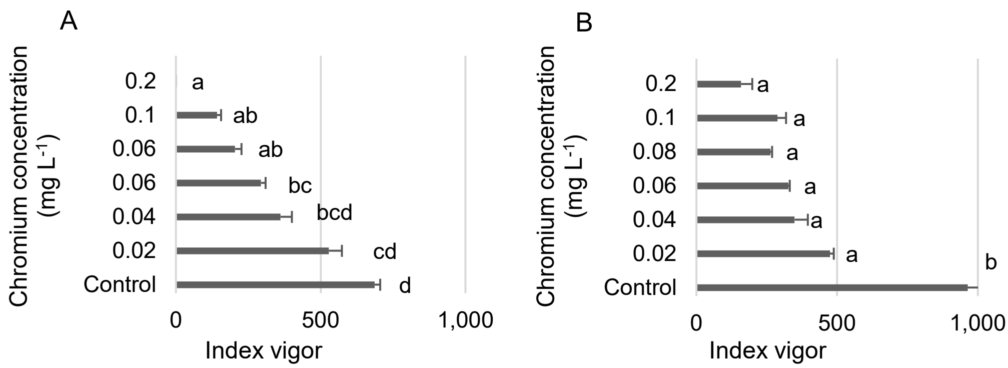

Figure 5. Effect of different chromium concentrations on the index vigor of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

In fact, the seeds treated with the concentrations 0.02 and 0.04 mg L^{-1}, have insigificant effect on their control. For the concentrations 0.06 to 0.2 mg L^{-1} chromium, presents a significant difference compared to the control (Fig. 5, A).

For lens, the vigour index law. It varied between 474.40 and 158.33 for the 0.02 and 0.2 mg L^{-1} chromium concentrations respectively, compared to 963.50 for the control (Fig. 5, B).

Overall, the vigour index of the lens seedlings are significantly different from the control (Fig. 5, B).

The negative effect of chromium is due to the reduction in radicle length and germination rates under this metal treatment.

Effect of chromium on the germination index of fenugreek and lens

The magnitude of phytotoxicity can be determined indirectly, since phytotoxicity decreases, the germination index increases (Komilis et al., 2005)

The addition of chromium solution on fenugreek at increasing concentrations reduces the germination index compared to the control (Fig. 6, A).

The germination index of fenugreek varies between 71.85 and 17.97% respectively for the concentrations 0.02 and 0.1 mg L^{-1} and dies at 0.2 mg L^{-1} of chromium (Fig. 6, A).

For fenugreek, the concentrations for the germination index, have a significant difference to control (Fig. 6.A). The same as, for lens, the concentrations from 0.02 to 0.2 mg L^{-1} of chromium are significantly different from the control (Fig. 6, B).

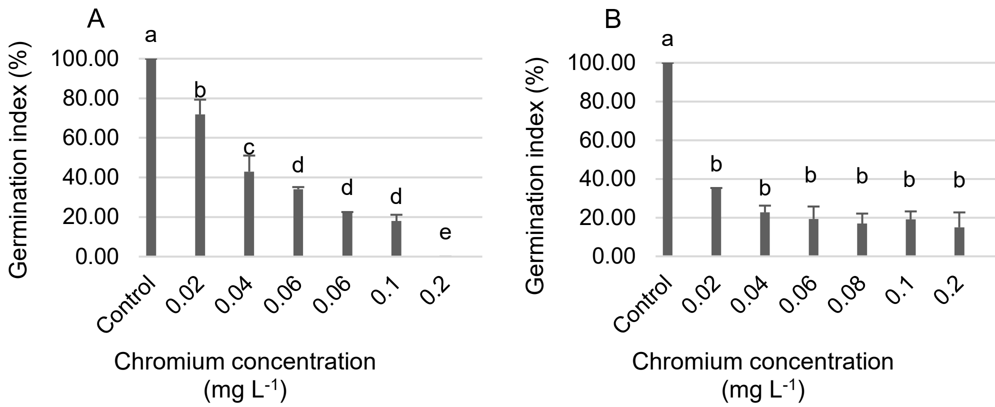

Figure 6. Effect of different chromium concentrations on the germination index of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

Seed germination, as the initial and critical stage of crop life cycle and the foundation of yield production (Nonogaki et al., 2018), is susceptible to chromium poisoning. And excellent seed germination is expressed in terms of both germination rate and subsequent seedling growth such as roots and shoots growth (Song et al., 2016).

(Joshi et al., 2019), found that Cr stress significantly inhibited the seed vigor index, radicle length, germ length and plant fresh weight of rice (*Oryza sativa* L.) and sorghum (*High Ghum Vulgare* L.). (Suthar et al., 2014) found that Cr decreased the growth of root and shoot of Mung bean (*Vigna radiata)* because the increase of the level of lipid peroxidation which is an indication of formation of elevated level of reactive oxygen species. (Mohammed et al., 2021) found that chromium also affected the germination rate of maize (*Zea mays .L*) by 20%.(Lei et al., 2021), found that the germination rate, coleoptile length, radicle number per plant and radicle length of wheat under Cr stress decreased significantly and suppressed germination by 16%, radicle dry weight by 35% and coleoptile dry weight by 24%.

Similar to the above results, the present study showed that Cr toxicity significantly decreased the seed germination rate and suppressed the growth of radicles, stem and leaves of germinated seeds. Probably, the parameters studied decrease, due to the measurement methods, that based primarily on the germination rate, radicles length and stem.Thus there is a reciprocal relationship between these studied parameters and tolerance index, root toxicity index, seeds vigor index and germination index of fenugreek and lens under stress of chromium.

CONCLUSION

Our results suggest that chromium affected germination of fenugreek and lens differently and depending on the used concentrations. Seed germination, lenght of radicles, stem and number of leaves, tolerance index, root toxicity index, seeds vigor index and germination index were extremely affected under chromium stress. Suggesting that fenugreek were highly sensitive. We could also conclude that lens was able to tolerate the increase of Cr concentrations showing low decrease of the measured characters. We can consider the toxicity is maximun and lethal at concentration 0.2 mg L^{-1}(when we depassed the maximum accepted accumulation in water 0.1 mg L^{-1} for water irrigation in Morocco).

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