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Full Length Research Paper

Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.)

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This study was conducted to determine and compare the inhibitory effects of chromium on seed germination and early seedling growth of melon (*Cucumis melo* L.). Chromium applications were controls; 2.5, 5, 10, 25, 50, 75, 100, 200 and 300 mgl^{-1} Cr in germination stage, and controls; 2.5, 5, 10, 20, 30, 40, 50, 60 and 70 mgl^{-1} Cr in early seedling stage. Excess chromium was limited to germination rate, germination index, mean germination time and germination uniformity index values in germination level. Radicle length, radicle fresh and dry weight, hypocotyll length, hypocotyll fresh and dry weight, growth tolerance index and seedling relative growth rate was negatively affected by the increased chromium concentrations at the seedling stage. Response of seedlings to chromium was more than that of seed germination. This event is based on the impermeability of seed coats and selectivity of embryos against chromium.

Key words: Chromium, germination, seedling, tolerance index, relative growth rates.

INTRODUCTION

Heavy metals are the intrinsic component of the environment with both essential and non essential types. It is usually accumulated due to unplanned municipal waste disposal, mining and use of extensive pesticides. Other agro-chemicals uses as chemical fertilizer are the significant cause of elevation in environment, and its persistence is the cause of most concern (Pandey and Pandey, 2008; Paksoy and Acar, 2009). Chromium (Cr) is a non-essential and toxic element to plants; Cr is found in all part of the environment including air, water and soil naturally occurring in soil. Normal range of Cr is from 10 to 50 mg/kg depending on the parental material (Pandey and Pandey, 2008).

Researchers have demonstrated experiments with plants associated with high levels of Cr. Thus, 1-5 ppm Cr present in the available form in the soil solution, either as Cr (III) of Cr (VI), is the critical level for a number of plant species. Cr, absorbed by plants grown in culture solutions, remained primarily in the roots and is poorly translocated to the leaves. Concentrations in plant tissues are associated with toxicity symptoms and are usually in the several hundred ppm range. Range of high Cr concentrations in plant tissues before toxicity symptoms was observed was from about 5 ppm for barley, corn, oats and citrus to 175 ppm for tobacco (Sinha et al., 2005).

Chromium interferes with several metabolic processes, causing toxicity to plants as exhibited by reduced seed germination or early seedling development (Sharma et al., 1995), root growth and biomass, chlorosis, photosynthetic impairing and finally, plant death (Scoccianti et al., 2006).

Fast and low costly applications are necessary for heavy metal contaminated agricultural areas as chromium. Since seed germination is the first physiological process affected by Cr, the capability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal. Also, there are different researches which show that early growth stages of seedling are very important indicators in determining toxicity impacts of heavy metals like chromium in plants (Sharma et al., 1995; Pandey and Pandey, 2008). This is because traditional cleanup processes of heavy metal contaminated soils are expensive and practical only in small areas; researches have looked for new cost effective

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Abbreviations: GUI, Germination uniformity indexes; **RL,** radicle length; **HL,** hypocotyl length; **RFW,** radicle fresh weight; HFW, hypocotyl fresh weight; **RDW,** dry weights of radicle; **HDW,** dry weights of hypocotyls; **GTI,** growth tolerance index; **RGR,** relative growth rates.

technologies that include the use of microorganisms, biomass, and live plants (Peralta et al., 2001).

Although there are some investigations (Wierzbicka and Obidzinska, 1998; Seregin and Kozhevnikova, 2005) about comparative mechanisms effect of heavy metals as chromium on seed germination and seedling growth-biomass in early growth stages, they are not adequate even though they were conducted with particular species. For this reasons, chromium effects must be exhibited in germination and early growth stages with different plant species. This study was conducted to determine and compare inhibitory effects of chromium on seed germination and early seedling growth of melon.

MATERIALS AND METHODS

Uniform seeds of *Cucumis melo* L. cv. Barada were used in two separate experiments as seed germination and early seedling. Seeds were surface sterilized with a 0.5% aqueous solution of sodium hypochlorite for 1 min to prevent fungal attack, and triple-rinsed in distilled water and dried between two paper towels for these experiments.

Germination experiments

Fifty seeds were placed in 90 mm petri dishes containing a double layer of paper towel as three replications, and were moistened with 5 ml of distilled water (control) of 2.5, 5, 10, 25, 50, 75, 100, 200 and 300 mgl^{-1} Cr solutions (as $CrCl_3.6H_2O$). The seeds germinated in the dark at 25 ± 2°C. Thereafter, germinated seed were counted and removed daily over a 20-day period when seeds exhibited radicle elongation of 2 mm.

Seedling experiments

The seeds were moistened with distilled water and were allowed to germinate until the radicles has elongated to 1 ± 0.1 cm in length in petri dishes. The germinated seedlings were replaced with a double layer of paper towel. Twenty-five seedlings were placed and grew in pot (15 x 9 cm) with 10 ml of distilled water (control) and 2.5, 5, 10, 20, 30, 40, 50, 60 and 70 mgl^{-1} Cr solutions (as $CrCl_3.6H_2O$).

Measurements

Germination index (G-ind) = ∑(Gt/Dt)

Where Gt = germination rate at day t; Dt = day t (Li et al., 2007).

Maximum germination rate or germination rate (G-max) = (G/T)*100

Where, G = number of total germinated seeds; T = number of total seeds in experiment.

Mean germination time (T-mean) = ∑(GtDt/G)

Where Gt = number of germinated seeds at day t; Dt = day t; G = germinated seeds.

Germination Uniformity Indexes (GUI) for GUI75-25 is the time between 25 - 75% and GUI90-10 is time between 10 - 90% of G-max or germinated seeds (Jalink and Van Der Schoor, 2000).

After 10 days of experiment, the radicle-hypocotyl length (RL and HL in mm) and fresh weight (RFW and HFW in mg) of the treated seedlings and the controls were measured. After drying for 48 h at 60°C in an oven, the dry weights of radicle and hypocotyls (RDW and HDW in mg) was obtained.

Growth tolerance index (GTI), which gives the integrated percentage of Cr-treated to Cr-untreated control seedling parts, gives opinion for effect of applied stress factor on plant growth and development. GTI was calculated using the established values of all morphological parameters. The changed formula below is used to obtain the equations of Rout et al. (2000) and Miteva et al. (2005)

$$GTI = \frac{1}{n} \sum_{i=1}^{n} \frac{PCr_i}{Pc_i} \times 100$$

Where, PCr_i is the established value of the respective *i*-th parameter of the nickel applied plants, Pc_i is the established value of the respective *i*-th parameter of the control plants, and n is the number of the used morphological parameters (radicle length-cm, radicle fresh and dry weight-g, hypocotyll length-cm, hypocotyll fresh and dry weight-g).

Relative growth rates were calculated with RGR = ln FV – ln IV / $t–t_0$; where RGR is the mean relative growth rate for elongation, fresh weight and dry weight of whole seedlings (radicle+ hypocotyll). FV is final value of any parameter at t (final time) and IV is initial value of any parameter at t_0 (initial time) (Hilbert et al., 1981).

Statistical analysis

Seed germination and seedling development experiments utilized a complete block design at each stage of the research with three replications. The data significances were analyzed by using analysis of variance and the differences between means treatments were compared by Duncan's multiple range test at $p < 0.05$. Data given in percentages were transformed to angular transformation values by arcsin square root before statistical analysis.

RESULTS

It is exhibited from the findings that melon seeds show deterioration after increased chromium treatment. The deterioration of seeds was observed in their low performance during the germination process and in poor development during the early seedling stages.

Analysis of variance (not shown) indicated that G-max and G-ind (Germination Index) was negatively affected by excess Cr concentrations in solution (Table 1). G-max was not affected in 2.5 and 5 mgl^{-1} Cr, although G-max reduced in 10, 25, 50, 75, 100, 200 and 300 mgl^{-1} Cr treatments relative to the control plants by 3.6, 5.7, 14.3, 19.3, 31.4, 38.6 and 44.3%, respectively ($p<0.001$). Increase in Cr concentrations of 2.5, 5, 10, 25, 50, 75, 100, 200 and 300 mgl^{-1}; and Cr reduced G-ind by 19.3, 34.7, 46.9, 56.4, 60.7, 70.1, 75.8, 78.8 and 82.1% of the control, respectively ($p<0.001$).

T-mean, T50, T75-25 uniformity index and T90-10 uniformity index were negatively affected at the $p < 0.001$ level by an increase in chromium concentrations (Figure 1). T-mean of seeds was reached at 1.22 to 2.99 fold of control in 2.5 to 300 mgl^{-1} Cr concentrations. T50 was not

Table 1. G-max (germination rate) and G-ind (germination index) in melon as affected by chromium concentrations.

Chromium (mg L-1)	G-max	G-ind
0	93.3a (75.3)	42.4a
2.5	90.7a (73.1)	34.2b
5	92.0a (74.4)	27.7c
10	90.0ab (71.8)	22.5d
25	88.0ab (70.2)	18.5e
50	80.0bc (63.7)	16.7e
75	75.3cd (60.3)	12.0f
100	64.0de (53.2)	9.3fg
200	57.3e (49.2)	9.0fg
300	52.0e (46.2)	7.6g
P	0.001	0.001
$LSD_{0.05}$	7.77	3.50

*Values enclosed in parentheses show arcsine-transformed means.

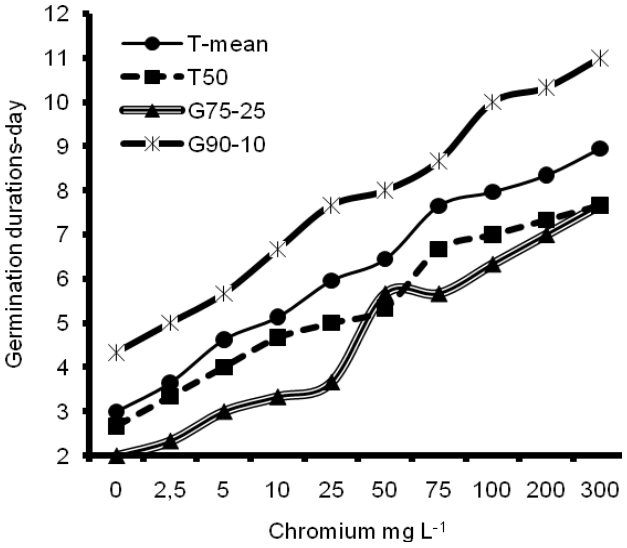

Figure 1. Effects of chromium concentrations (mg l^{-1}) on germination rate (T = mean: $p < 0.001$, $LSD_{0.05}$: 0.7), germination half time (T50: $p < 0.001$, $LSD_{0.05}$: 1.2), uniformity index for 75-25 (UI75-25: $p < 0.001$, $LSD_{0.05}$: 1.3) and uniformity index 90-10 (UI90-10: $p < 0.001$, $LSD_{0.05}$: 1.6) in melon.

affected in 2.5, 5 and 10 mgl^{-1} Cr, but it was delayed in 25, 50, 75, 100, 200 and 300 mgl^{-1} Cr applications of 1.88, 2.00, 2.50, 2.63, 2.75 and 2.88 times of control. Germination uniformity indexes, time between 25 - 75% (UI75-25) and 10 - 90% (UI90-10) of G-max, displayed increased trend due to elevated chromium. The UI75-25 showed slow germination at elevated concentrations of Cr while UI75-25 increased significantly from 117 to 383% and 2.5 to 300 mgl^{-1} Cr concentrations by comparison control. UI90-10 of seeds was not affected in 2.5mgl^{-1} Cr, but it was significantly delayed in solutions containing 5 to 300 mgl^{-1} Cr from 131 to 254% of the control.

The statistical analysis at $p<0.001$ level show that Cr reduced radicle elongation and hypocotyll length (Figure 2). In solutions containing 2.5 to 70mgl^{-1} Cr, radicle elongation significantly declined from 14.7 to 58.5% of the control. The elevated chromium at 2.5 to 70mgl^{-1} induced a decrease in the hypocotyll height in melon seeds from 11.4 to 52.7%, compared with the control. Excess chromium treatments (2.5 to 70 mgl^{-1} Cr) depressed radicle fresh biomass and hypocotyll fresh biomass in melon seedlings at the $p<0.001$ (Figure 3) and in Cr concentrations such as 2.5 to 70 mgl^{-1} Cr, radicle fresh weight increased by 89 to 49% of the control. In accordance with control treatments, elevated doses of chromium, 2.5 to 70mgl^{-1}, declined the hypocotyll fresh biomass 6.0 to 42.9%, respectively. Chromium in melon prevented the radicle (except 2.5 and 5 mgl^{-1} Cr) and hypocotyll dry biomass (except 2.5, 5 and 10 mgl^{-1} Cr) at $p<0.001$ significance level (Figure 4). Radicle dry weight decreased by 12.4 to 65.1% in 10 to 70mgl^{-1} Cr compared to the control. Also, hypocotyll dry biomass was 35.8 to 50.8% of the control by 20 to 70mgl^{-1} chromium applications, respectively.

Analysis of variance detected that the GTI of melon seedlings was calculated based on morphological parameters and showed inhibition of growth and biomass synthesis in all Cr concentrations at $p<0.001$ (Figure 5). The value of GTI was 100 in control while it changed from 91.59 to 46.65 in 2.5 to 70mgl^{-1} Cr, respectively.

When compared with control, all RGR values of length and fresh-dry weight in whole seedlings of melon was negatively affected by elevated chromium at $p<0.001$ (Figure 6). The RGR-L showed decreasing prolongation from 4.8 to 28.1% and RGR-FW decreased significantly from 2.4 and 17.6% in 2.5 to 70 mgl^{-1} Cr concentrations of control. RGR-DW values was not affected from 2.5, 5 and 10 mgl^{-1} Cr, but these values decreased from 12.1 to 25.6% in 20 to 70 mgl^{-1} Cr of control.

DISCUSSION

This investigation showed that germination and viability of seeds were negatively affected by elevated chromium concentration. Excess chromium applications significantly inhibited germination properties like G-max, G-ind and all germination durations (T-mean, T50, UI75-25 and UI90-10) which were important seed germination parameters. There are reports on the inhibitory effect of elevated concentrations of chromium seed germination for different species: chromium reduced the germination from 15 to 55% in alfalfa at 5 to 40 mgl^{-1} Cr (Peralta et al., 2001); from 2.2 to 100.0% in celery at 0.01 to 10 mM Cr (Scoccianti et al., 2006); and 17 to 44% in pea at 25 to 100 mgl^{-1} Cr (Pandey and Pandey, 2008) in comparison with control applications. Samantaray (2002) compared Cr-tolerant and Cr-sensitive cultivars of mung bean in 0,

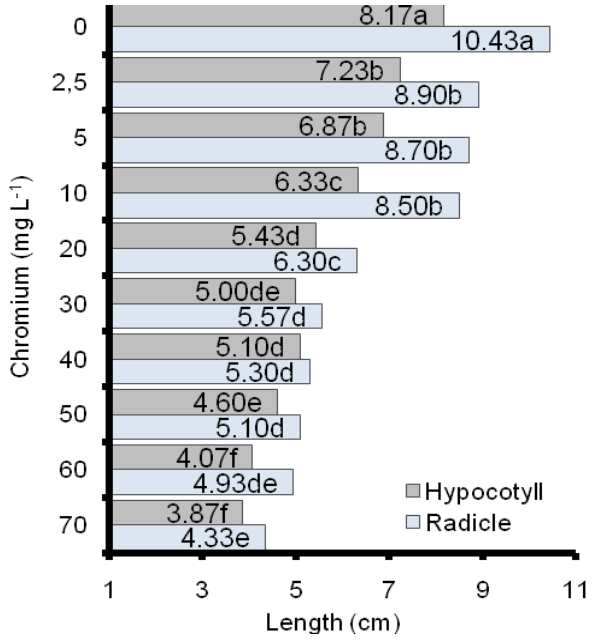

Figure 2. Effect of chromium on length of radicle ($p < 0.001$, $LSD_{0.05}$: 0.60) and hypocotyll ($p < 0.001$, $LSD_{0.05}$: 0.43) in melon.

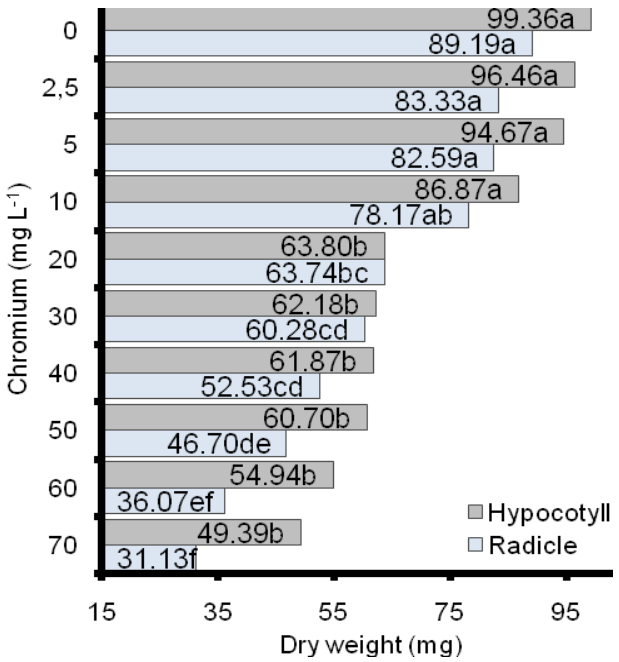

Figure 4. Effect of chromium on dry weight of radicle ($p < 0.01$, $LSD_{0.05}$: 14.75) and hypocotyll ($p < 0.01$, $LSD_{0.05}$: 15.28) in melon.

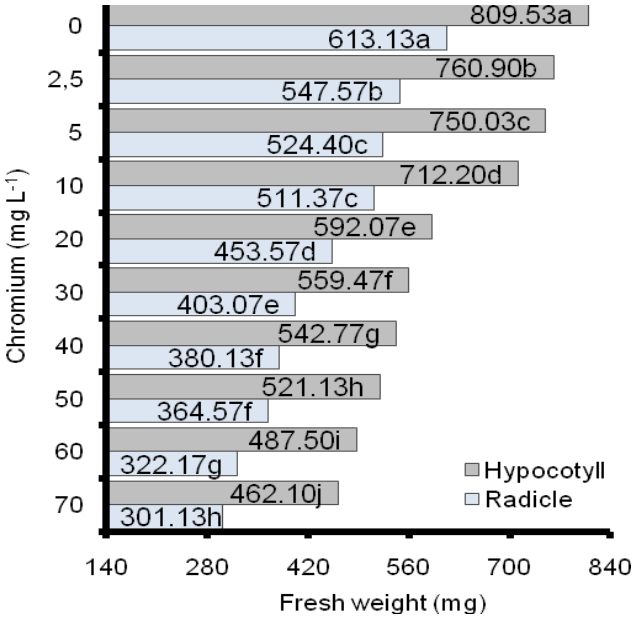

Figure 3. Effect of chromium on fresh weight of radicle ($p < 0.001$, $LSD_{0.05}$: 19.16) and hypocotyll ($p < 0.001$, $LSD_{0.05}$: 4.78) in melon.

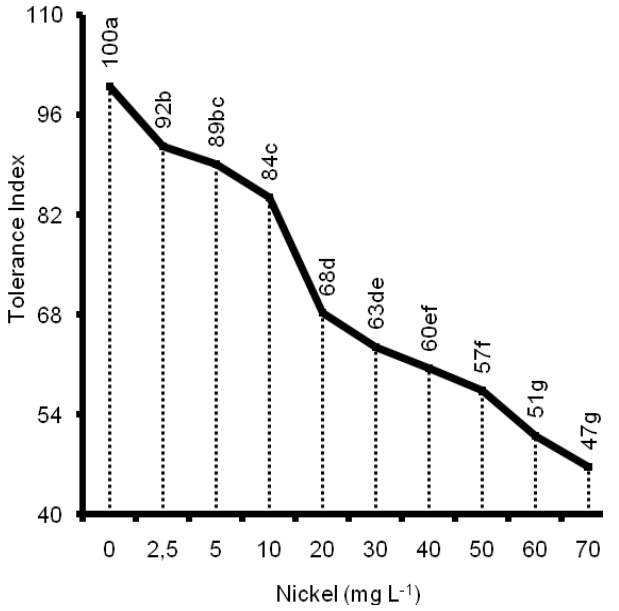

Figure 5. Growth tolerance index (GTI: $p < 0.001$, $LSD_{0.05}$: 5.13) in melon by affected excess chromium.

24, 48, 96 and 192 µM Cr. The results showed that low concentration of chromium (24 µM) did not affect seed germination but low percentage germination was recorded in the case of Cr-sensitive cultivars grown on 96 and 192 µM of chromium. Sharma et al. (2005) conducted a study involving the growth of spinach variety 'Punjab Green' in a greenhouse on silty clay loam and sandy soils equilibrated with different levels of applied Cr (0, 1.25, 2.5, 5, 10, 20, 40, 80, 160, and 320 mg Cr kg^{-1} soil). It was observed that there was no germination of spinach

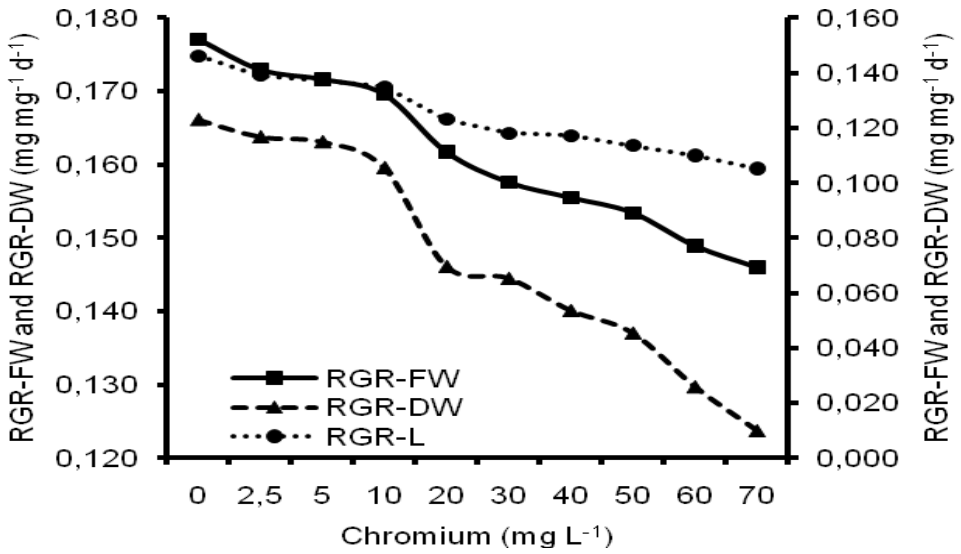

Figure 6. RGR-Length (RGR-L: $p < 0.001$, $LSD_{0.05}$: 0.0034), RGR-fresh weight (RGR-FW: $p < 0.001$, $LSD_{0.05}$: 0.0012) and RGR-dry weight (RGR-DW: $p < 0.001$, $LSD_{0.05}$: 0.0117) values of whole seedlings in melon by affected excess chromium.

when Cr at 320 mg Cr kg^{-1} rate was applied in silty clay loam soil and at 40 mg Cr kg^{-1} rate in sandy soil due to Cr toxicity. Other researches showed that germination of rice cultivars was effectively reduced from 0.86 to 100%, and from 10-800 ppm of Cr concentrations in culture medium (Gyawali and Lekhak, 2006).

Chromium significantly reduced the radicle length and hypocotyll length of tomato seedlings, and their inhibited level increased with the increase of Cr concentration. Also, Cr had significant inhibition effect on fresh and dry weight of radicle and hypocotyll. This is because growth limits in seedlings parts, relative growth rate and tolerance index were also affected by elevated chromium concentrations. Similar data were observed by different researchers. An experiment was carried out to study plant responses to the addition of 0, 10, 50, and 100 $mgkg^{-1}$ of chromium, respectively, and radish was used as plant test. Radish was sown after 25 days of incubating the soil with Cr. In Cr, dry matter yield of roots and shoots showed greater values, but these tissues reduced in 100 mg kg^{-1} when Cr(VI) was applied (Fernandez et al., 2002). Seven plant species (Indian mustard, canola, clover, soybean, sunflower, tobacco and loblolly pine) were investigated for chromium tolerance at 1 mM, and relative stem extension ratio was one of the test parameter. Chromium reduced the parameter of all the species (Mei et al., 2002). Chromium concentrations at 20, 40 and 80 mgl^{-1} by control reduced the lengths and dry biomass of roots and shoots in *Convolvulus arvensis* (Gardea-Torresdey et al., 2004). In the rice cultivars, Gyawali and Lekhak (2006) studied root and shoot growth, fresh and dry weight, and the relative degree of toxicity of Cr (VI). Increased Cr (VI) concentration of 10-800 mgl^{-1} in culture medium led to the detection of inhibited growth parameters. There was a reduction in growth, dry weight and vigour index in four soybean genotypes of soybean at 5 - 200 mgl^{-1} concentrations of chromium, according to control application (Ganesh et al., 2009).

Chromium concentrations were used more in germination stage (max 300 mg L^{-1} Cr) than in seedling stage (max 70mgl^{-1} Cr). In other words, tomato was more sensitive in seedling stage than in germination stage for elevated chromium. Our results were supported by Gyawali and Lekhak (2006), they noted that an increase in Cr (VI) concentration 10-800 mg L^{-1} in culture medium negatively limited the seedling growth of rice cultivars. These results were confirmed by Jun et al., (2009) who investigated effects of 0 to 3.3mM Cr on germination and early seedling growth of six pulses and observed that root elongation and coleoptile growth of six pulse plants were more sensitive than seed germination. This is because seed is a stage in the plant life cycle that is well protected against various stresses. However, soon after inhibition and subsequent vegetative developmental processes, they become stress-sensitive in general. Therefore, seeds are thought to carefully monitor such external parameters as light, temperature and nutrient in order to maintain the protective state until external conditions become favorable for the subsequent developmental processes. Although such critical regulatory mechanisms are likely to operate in seeds at the onset of inhibition, little is known about how stress tolerance is modulated at different phases of germination (Li et al., 2005).

This event can be expressed that barrier role of seed coat were as a response of embryo to chromium. Thus, heavy metal tolerance started with seed coat in plants. Seed germination is tolerant to heavy metals apparently because the seed coat can be impermeable to metal

ions. There is relationship between the metal intakes of seed and media water status. These indicate that the seed coat was only very slightly permeable to metal (lead) in the first period of inhibition, during the period of intensive water uptake. In contrast, during the last period, when water uptake slows, the seed coats become much more permeable to metal (lead) (Wierzbicka and Obidzinska, 1998). Embryo plays a role in selective penetration of different heavy metals into seeds. This was first suggested by the fact that seeds still germinated in the presence of high concentrations of heavy metals, but subsequent seedling growth (after the breakage of seed coat) was severely inhibited at much lower concentrations of these heavy metals. Isolated embryos were much more sensitive to heavy metals than intact seeds (Li et al., 2005). When metals are absorbed by the embryo tissues, they may exert the toxic effect on the embryo radicle and in this way inhibit root growth after protrusion. The effect of heavy metals on seed germination relies on their ability to reach embryo tissues across the physiological barriers, predominantly, the seed coats. This ability directly depends on the seed coat structure which varies in diverse plant species and on the physical and chemical properties of the metal ions themselves (Seregin and Kozhevnikova, 2005).

The result of this study showed that increase concentrations of chromium inhibited seed germination and limited seedling growth. Seedling growth is more sensitive to chromium stress than seed germination in melon. This is because, it can be said that seed coats impermeability and embryos selectivity affected the tolerance of chromium impacts. This event can be used practically and cheaply for selection of tolerant species or chromium varieties during the germination and early seedling stages.

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