Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2015, 7(3):322-329. DOI: 10.15835/nsb.7.3.9563

AcademicPres

Notulae Scientia Biologicae

# The Effect of Halopriming and Salicylic Acid on the Germination of Fenugreek (*Trigonella foenum-graecum*) under Different Cadmium Concentrations

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**Abstract**

The hereby study was based on a factorial experiment conducted in a completely randomized design with four replications, at Agriculture College, Shahrekord University, Iran, in 2014. The role of salicylic acid (SA), potassium nitrate ($KNO_3$) and potassium chloride (KCl) was evaluated on seed germination of fenugreek (*Trigonella foenum-graecum* L.) under different cadmium concentrations. Treatments included four levels of seed priming (no priming, potassium chloride, potassium nitrate, salicylic acid) and four levels of cadmium concentration (0, 10, 20, 30 mg/L). Cadmium chloride caused a significant inhibition in germination percentage, root elongation, shoot elongation and seedling dry weight. The shoot length was more sensitive to cadmium concentrations than the root length. Primed seeds with SA (100 mg/L) proved protection against Cd stress and increased the germination percentage, root elongation, shoot elongation and dry weight of seedlings compared to the control treatment. Seeds treated with SA alleviated the Cd negative effect on germination parameters. In conclusion, using seed priming with salicylic acid can be recommended as a good technique for fenugreek crop on fields exposed to high cadmium toxicity.

***Keywords:*** heavy metal, seedling, seed priming, root, vigor

## Introduction

Cadmium (Cd) is a highly toxic heavy metal, representing an environmental concern, because of its low mobility in soils (D'Souza *et al.,* 2008). It can be accumulated in soil through agricultural application of sewage sludge, fertilizers, and/or through land disposal of Cd-contaminated municipal and industrial wastes (D'Souza *et al.,* 2008). Cd causes oxidative stress (Mudipalli, 2008) by formation of free radicals. Oxidative stress refers to enhanced generation of reactive oxygen species (ROS), that may result from alterations of numerous physiological processes caused at cellular/molecular level by inactivating enzymes, blocking functional groups of metabolically important molecules (La Rocca *et al.,* 2009); it can also overwhelm cell's intrinsic antioxidant defense and can lead to cell damage or plant death (Krystofova *et al.,* 2009) and disrupting membrane integrity (La Rocca *et al.,* 2009).

Cadmium competes with iron, manganese, zinc, copper, calcium, magnesium and potassium (Liu *et al.,* 2003), therefore this metal has high priority for removal from aqueous environments (Rama *et al.,* 2002).

Cadmium toxicity alters the morphological, physiological, biochemical and structural processes of plants, including inhibition of germination and seedling growth (Mishra *et al.,* 2006). After long-term exposure to Cd, roots become mucilaginous, browning, and decomposing; therefore, a reduction of shoots can be observed, along with root elongation. Cd was found to inhibit lateral root formation, while the main root became brown, rigid and twisted (Rascio and Navari-Izzo, 2011). The main reason indicated was disorder of cell division and abnormal enlargement of epidermis and cortical cell layers in the apical region (Liu *et al.,* 2010).

The plant root is the first area affected by cadmium, so the reaction with root cells leads to changes in the physiological properties of cell plasma membranes, having high ability to cross cell membranes rooted (Astolfi *et al.,* 2003).

Fenugreek *(Trigonella foenum-graecum* L.) belongs to Leguminosae (Fabaceae) family (Kaviarasan *et al.,* 2007). This is a native crop extending from Iran to northern India (Petropoulos, 2002) and it is commonly used as a condiment in food preparation for its nutritive and restorative properties. It was also known and used in folk medicine for centuries for a wide range of diseases including diabetes (Eidie *et al.,* 2007).

Stand establishment is of primary importance for optimizing field production of any crop plant. At sub-optimal environmental conditions, poor seed germination and subsequently poor field establishment is a common phenomenon (Mwale *et al.,* 2003). Seed priming is a pre-sowing strategy for influencing seed germination and seedlings' development by modulating germination metabolic activity prior to emergence of the radical, and generally enhances germination rate and plant performance (Bradford, 1986). This technique changes the pattern of N and $Ca^{+2}$

*Received: 27.03.2015. Received in revised form: 05.09.2015. Accepted: 08.09.2015. Published online: 24.09.2015.*

homeostasis, both of the seeds and seedlings; these modifications were associated with enhanced α-amylase activity and the content of reducing sugars. Positive correlation of seedling attributes with the nutrient content suggested that, as a result of seed priming, most N and $Ca^{+2}$ were partitioned to the embryo, enhancing seedling emergence and subsequent growth of rice seedling (Farooq *et al.,* 2010).

Applying priming treatments improved the antioxidant activity of treated seeds (Wang *et al.,* 2003). Priming can facilitate the growth of the embryo before germination, subsequent embryo development and improves germination and emergence after planting under normal, as well as stress, conditions (Neamatollahi *et al.,* 2009). The researchers also expressed uniform seedlings from seed osmo-priming, which is supposed to be due to a more uniform and more rapid synthesis of proteins (El-Araby and Hegazi, 2004).

Salicylic acid (SA) is involved on membrane permeability, absorption of ions and prevention of both biotic (Alvarez, 2000) and abiotic stresses (Tissa *et al.,* 2000). It can affect the accumulation of heavy metals, as observed in pea plants for which soaking of seeds in SA resulted in reduced accumulation of Cd in plants (Popova *et al.,* 2009).

Cadmium induces changes in physiological, biochemical and morphological processes such as oxidative stress, reduction of the germination percentage, reduction in the root length and shoot length and seedling weight. However, progress of industry makes pollution of water sources increasing with heavy metals, such as cadmium, while on the other hand, for farming systems, use of water resources is essential. Germination is known to be one of the most important and most critical steps, influenced by changes in the surrounding environment. The present study was performed to determine the effect of seed priming treatments in reduction of cadmium toxicity on seed germination sensitive plants, like fenugreek seed.

## Materials and Methods

### *Seed priming*

In order to evaluate the effect of seed priming on the germination and seedlings' growth traits of fenugreek (*Trigonella foenum-graecum* L.) under cadmium stress, a factorial experiment was conducted in completely randomized design with four replications, at the Faculty of Agriculture, Shahrekoord University, Iran, in 2014. The fenugreek plants were purchased from Pakan Bazr Company, Isfahan, Iran. Seeds were rinsed with water and distilled water, then surface sterilized in 40% sodium hypochlorite (vol/vol) for 10 minutes, followed by 70% alcohol (vol/vol) for 10 seconds; after, were thoroughly rinsed with sterile deionized water and then seeds were primed.

For halopriming, seeds were placed on -0.3 MPa of potassium nitrate ($KNO_3$) and -0.3 MPa potassium chloride (KCl), while for hormopriming, they were placed on a salicylic acid (SA) solution (100 mg/L) in the chamber room, under dark condition at 15 °C, for 24 hours. As a next step, seeds were rinsed with distilled deionized water ($ddH_2O$) three times, to wash off the $KNO_3$, KCl and SA solutions from their surface, than air dried for 24 hours at 25 °C to reach the original moisture content (~12–13%) and immediately used for germination tests. Unprimed seeds were used as control.

The amount of potassium nitrate required was calculated using the Want hoof equation (Siebert and Richardson, 2002):

Ψ s = -miRT

Ψ s = Osmotic potential (MPa)

m= Molality of solution (mol dissolved in 1000 g water)

i= Ion coefficient (equal to 2 for salts dissolved in water)

R= Fixed amount of gas (00831/0 liter MPa per mol K)

T= Temperature in degrees Kelvin = temperature in degrees Celsius + 273.

### *Germination of seeds under cadmium conditions*

For germination test, unprimed seeds were surface sterilized following the same steps as for primed seeds. A total of 200 seeds (primed and unprimed) in four replicates (50 seeds/replicate) for each treatment, were sown within 9-cm-diameter Petri dishes on filter paper soaked with 5 mg/L $CdCl_2$, at concentrations of 0, 10, 20 and 30 mg/L $CdCl_2$, constituted an experimental unit for all experiments. Seeds were incubated in a seed germinator, at alternate temperatures ranging from 20-30 °C, under dark conditions. Seeds were considered germinated when at least 2 mm long radicle protruded through the seed coat (ISTA, 2009).

As a final step, after 22 days germination percentage, germination rate, root and shoot length, shoot dry weight (DSW), radicle dry weight (DRW) and vigor index (VI) were recorded to evaluate germination performance. Daily germination percentage was recorded and subjected to statistical analysis. The root and shoot vigor were calculated as the sum of total root length (cm) and shoot length (cm) of all the seedlings of a replicate divided by the number of seedlings. Fresh root and shoot were then placed in a hot air oven (70 °C for 24 hours) to dry (ISTA, 2009). Root and shoot dry mass were measured with electrical balance.

Germination percentage (GP) was calculated using the Equation 1 (Ikic *et al.,* 2012):

$$GP = \frac{\textit{Total seeds germinated after day 14}}{\textit{Total number of planted seeds}} \quad \text{(Equation 1)}$$

Germination rate (T50) was defined as days needed to reach 50% of GP.

Coefficient of velocity of germination (CVG) was calculated using the Equation 2 (Kotowski, 1926):

$$CVG = \frac{Nt}{\sum nt} \times 100 \quad \text{(Equation 2)}$$

In this regard, CVG, N and n are the speed of germination, total germination at the end of the experiment and germinated seeds at time t, respectively.

The vigor index (VI) was calculated as the product of radicle and shoot length by germination percentage (Abdul-Baki and Anderson, 1973).

Seed vigor index was calculated by the Equation 3 (Kalsa and Abebie, 2012):

$$VI = \frac{GP\%}{RL(cm)} \quad \text{(Equation 3)}$$

*Statistical analysis*

Normality of data was checked prior to analyzing. Data were analyzed statistically by using analysis of variance with SAS 9.1 software. Data were analyzed by using both one- or two-way analysis of variance (ANOVA) and mean comparisons were performed by LSD test; F-test was calculated ($p < 0.05$) to determine whether differences among means were significant between treatments within halopriming, hormopriming and cadmium stress respectively.

## Results

*Germination percentage*

Analysis of variance in this experiment showed that seed priming, cadmium concentration and interaction between priming and cadmium concentration had significant effects ($p \geq 0.01$) on the germination percentage, germination rate, root length and shoot length (Table 1). Germination percentage was significantly decreased with increasing the concentrations of cadmium compared to control treatment. Exposure to 30 mg/L of cadmium, salicylic acid, potassium nitrate and potassium chloride caused an increase of the germination percentage by 4.16, 1.04 and 2.08%, respectively compared with the control. KCl and $KNO_3$ showed significant difference with the control, not being phenotypically noticeable though. The highest germination percentage was obtained for primed seeds with 100 mg/L SA in all cadmium concentrations compared to other treatments. The lowest germination was observed in the control treatment using 30 mg/L cadmium (Fig. 1). $KNO_3$ treatment had no significant difference with control treatment in cadmium concentration 10 mg/L, while 0 mg/L Cd concentration had negative effect on germination percentage compared to control treatment. Exposure to 20 and 30 mg/L of cadmium, $KNO_3$ and KCl solutions resulted in no significant effect, but the values obtained were located in higher level than control treatment (Fig. 1).

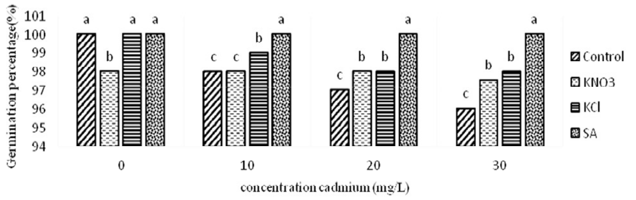


Fig. 1. Germination percentage of unprimed (UPS) and primed (PS) seedlings of fenugreek (*Trigonella foenum-graecum* L.) under different Cd concentrations

Germination percentage was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 hours. The unprimed seeds were used as the control. Means with similar letters are not significantly different ($p \leq 0.05$) based on LSD test.

Table 1. Analysis of variance (mean square) for the effects of seed priming on the germination rate, germination percentage, shoot length and root length, of fenugreek seeds under different cadmium concentrations

| Source of variance | Df | Germination percentage | Germination rate | Shoot length | Root length |
|---|---|---|---|---|---|
| Seed priming (P) | 3 | 17.22** | 2211.9** | 9.62** | 32.15** |
| Cadmium (Cd) | 3 | 7.89** | 46.46** | 12.30** | 8.33** |
| P × Cd | 9 | 2.56** | 90.98** | 1.41** | 0.407** |
| Error | 48 | 0.22 | 5.05 | 0.0030 | 0.0037 |
| C.V. (%) | | 0.48 | 2.53 | 1.41 | 1.64 |

** Significant at 0.01 probability level

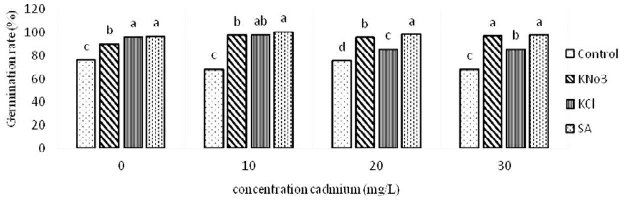


Fig. 2. Germination rate of unprimed (UPS) and primed (PS) fenugreek seedlings (*Trigonella foenum-graecum* L.) under different Cd concentrations

Germination rate was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 hours. The unprimed seeds were used as the control. Means with similar letters resulted in no significant different ($p \leq 0.05$) based on LSD test.

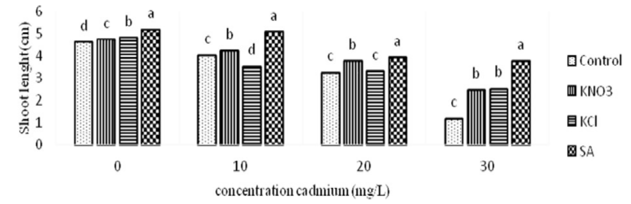


Fig. 3. Shoot length of unprimed (UPS) and primed (PS) seedling of fenugreek (*Trigonella foenum-graecum* L.) under different Cd concentrations

Shoot length was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 hours. The unprimed seeds were used as the control. Means with similar letters were not significantly different ($p \leq 0.05$) based on LSD test.

*Germination rate*

There was a significant ($p < 0.01$) effect of priming treatments on the germination rate (GR). Treated seeds in all cadmium concentrations had higher germination rate than the control. At 30 mg/L of cadmium, germination rate was increased by 43.51%, 42.7% and 26.26% with salicylic acid, potassium nitrate and potassium chloride compared to control, respectively. The maximum and minimum GR were observed for the primed seeds with SA and control, respectively. There was no significant difference between KCL and SA solution for GR in 0 and 10 mg/L cadmium. However, $KNO_3$ and SA solution had no significant difference for GR in 30 mg/L cadmium. Seeds primed using $KNO_3$ showed higher germination rate than KCl treatment, at 20 and 30 mg/L cadmium concentrations. Seeds exposed to 10 mg/L cadmium had no significant differences between them, but $KNO_3$ soluble was lower than KCl soluble in cadmium concentration 0 mg/L (Fig. 2).

*Shoot length*

Interaction between salicylic acid and cadmium was significant for root and shoot length (Table 1). In all concentrations of cadmium, the greatest shoot length was obtained with SA primed seeds and the lowest shoot length was obtained within the control treatment. However, control treatment was not significantly different with KCl treatment under 20 mg/L cadmium. $KNO_3$ and KCl treatments lead to shoot length increase compared to control treatment, except of shoot length for the treatment with 10 mg/L Cd in KCl solution which was lower than the control treatment. In all concentrations, there were significant differences among SA and

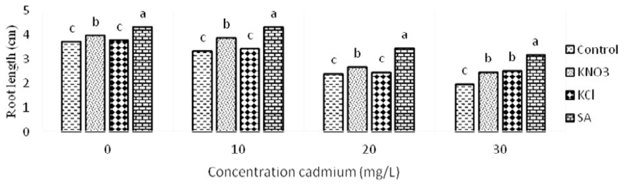


Fig. 4. Root length of unprimed (UPS) and primed (PS) fenugreek seedlings (*Trigonella foenum-graecum* L.) under different Cd concentrations

Root length was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 hours. The unprimed seeds were used as the control. Means with similar letters were not significantly different ($p \leq 0.05$) based on LSD test.

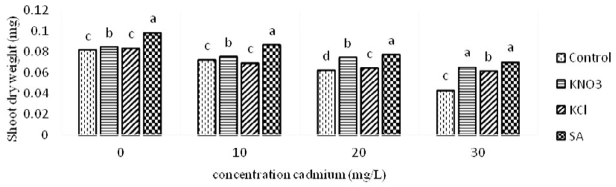


Fig. 5. Shoot dry weight of unprimed (UPS) and primed (PS) seedling of fenugreek (*Trigonella foenum-graecum* L.) under different Cd concentrations

Shoot dry weight was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 h. The unprimed seeds were used as the control. Means with similar letters were not significantly different ($p \leq 0.05$) based on LSD test.

other treatments for shoot length (Fig. 3). Exposed to 10 and 20 mg/L Cd, treated seeds with potassium nitrate showed a better shoot length development than those subjected to potassium chloride treatment. In 10 and 20 mg/L cadmium, the shoot length in case of KCl treatment was shorter than the control (Fig. 3). Thus, in control group (0 mg/L of Cd) KCl solution was located in a higher level than the treatment of $KNO_3$. Finally, at a concentration of 30 mg/L of Cd, there was no significant difference between halopriming treatments (KCl and $KNO_3$). Shoot growth decreased more than root growth in 30 mg/L cadmium. Exposed to 30 mg/L of cadmium, the shoot length increased by 214.16, 100 and 109.16% with salicylic acid, potassium nitrate and potassium chloride, respectively, in comparison with the control. This indicated the shoot length was strongly influenced by priming treatments and it increased more than other germination parameters. Also, salicylic acid proved to be the most effective among priming treatments.

*Root length*

Seed priming treatments showed different effects on the root length. It decreased significantly with the increase of cadmium level as compared to the control treatment. The highest root length (RL) was obtained from seeds primed with SA treatment, which indicated that SA pretreatment increased cadmium tolerance in fenugreek seeds. Lowest RL was attained from control in all concentrations of cadmium. There was no significant effect in the concentration of 0, 10 and 20 mg/L cadmium for KCl treatment. $KNO_3$ treatment in all concentrations of cadmium had most of root length than of control. Significant effects were observed between SA treatment and other treatments in all cadmium concentrations (Fig. 4). Salicylic acid, potassium nitrate and potassium chloride increased the root length by 61.85%, 24.74% and 41.23%, respectively, at a 30 mg/L cadmium concentration. Highly significant effect was observed between any of the treatments and control, while at concentrations of 0, 10 and 20 mg/L cadmium root length was not significantly affected compared with control, in the treatment of KCl (Fig. 4). Exposed to 0, 10 and 20 mg/L of Cd, seeds primed by $KNO_3$ treatment showed better root length development than KCl treatment. However, there was no significant effect between halopriming (KCl and $KNO_3$) treatments, at 30 mg/L Cd concentration (Fig. 4).

*Shoot dry weight*

Analysis of variance in this experiment showed that seed priming, cadmium concentration and interaction between priming and cadmium concentration had significant effects ($p \geq 0.01$) on the shoot dry weight, root dry weight and vigor index (Table 2). In primed and unprimed seeds, shoot dry weight

Table 2. Analysis of variance (mean square) for effects of seed priming on, root dry weight, shoot dry weight and vigor index of fenugreek under different cadmium concentrations

| Source of variance | df | Shoot dry weight | Root dry weight | Vigor index |
|---|---|---|---|---|
| Seed priming (P) | 3 | 0.0008** | 0.0000**4 | 331799** |
| Cadmium (Cd) | 3 | 0.0022** | 0.0002** | 85054** |
| P × Cd | 9 | 0.00008** | 0.00001** | 4236** |
| Error | 48 | 0.0000008 | 0.0000006 | 32.40 |
| C.V (%) | | 1.27 | 4.89 | 1.53 |

** Significant at 0.01 probability level

decreased significantly with the increased cadmium concentration compared to the control (Fig. 5). Salicylic acid, potassium nitrate and potassium chloride increased the shoot dry weight with 62.79%, 51.16% and 41.86%, respectively, at a concentration of 30 mg/L of cadmium. Seeds treated with 100 mg/L of SA produced maximum shoot dry weight compared to other treatments. However, $KNO_3$ and SA treatments had no significant effect at the concentration of 30 mg/L cadmium. The lowest shoot dry weight was observed in control treatment, in all concentrations of cadmium. At $KNO_3$ and KCl treatments, shoot dry weight was more improved than control, in all cadmium concentrations, except at the 0 and 10 mg/L concentrations that, had no significant difference between control and KCl treatments (Fig. 5). At all cadmium levels, soluble $KNO_3$ showed greater shoot dry weight than KCl treatment.

*Root dry weight*

Root dry weight decreased significantly with the increased of cadmium levels as compared to the control (Table 2). According to the results in Fig. 5, at all cadmium concentrations, maximum root dry weight was achieved from seeds primed with 100 mg/L SA, so that it had significant effect compared with control and the rest of the treatments. Minimum dry weight of root was observed in seeds primed with KCl at the 30 mg/L cadmium level. $KNO_3$ and KCl treatments had no significant effect than control, while the data for the concentration of 20 mg/L cadmium showed that root dry weight was higher than the control (Fig. 6). Salicylic acid, potassium nitrate and potassium chloride increased the root dry weight by 100%, 30% and 50%, in cadmium concentration of 30 mg/L. (Fig. 6). Exposed to 20 mg/L of cadmium, $KNO_3$ treatment showed higher root dry

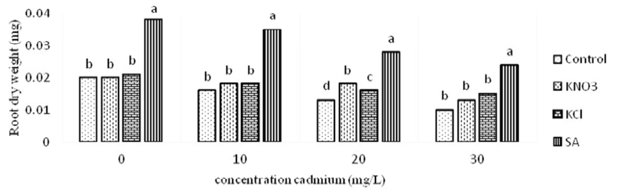


Fig. 6. Root dry weight of unprimed (UPS) and primed (PS) seedling of fenugreek (*Trigonella foenum-graecum* L.) under different Cd concentrations

Root dry weight was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 h. The unprimed seeds were used as the control. Means with similar letters were not significant different ($p \leq 0.05$) based on LSD test.

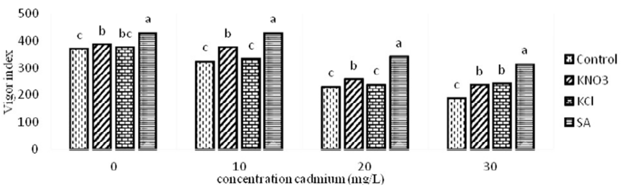


Fig. 7. Vigor index of unprimed (UPS) and primed (PS) seedling of fenugreek (*Trigonella foenum-graecum* L.) under different Cd concentrations

Vigor index was measured in unprimed seeds and when seeds were primed by 100 mg/L SA and -1 MPa $KNO_3$ and KCl solution for 24 h. The unprimed seeds were used as the control. Means with similar letters were not significantly different ($p \leq 0.05$) based on LSD test.

weight than KCl treatment. However, there was no significant difference between $KNO_3$ and KCl treatments in 10, 30 mg/L of Cd and control group (0 mg/L of Cd) (Fig. 6).

*Vigor index*

The maximum seedlings' vigor index was obtained in seeds primed with 100 mg/L SA solution (Fig. 7), while the minimum value was observed in the control treatment with 30 mg/L cadmium. In all concentrations of cadmium, seedlings' vigor of SA showed more significant effects than other treatments. There was no significant difference between KCl solution and control treatment in all cadmium concentrations, except in the case of 30 mg/L of cadmium, where seeds primed with KCl treatment showed higher vigor index compared to control. At all cadmium concentrations, $KNO_3$ treatment was located in higher level than KCl treatment, even though no significant effect was noted between them in 30 mg/L of Cd and control treatment (0 mg/L of Cd) (Fig. 7).

Improvement of vigor index in salicylic acid treatment resulted by same effects of the root length and germination percentages, however in potassium nitrate and potassium chloride treatment, root length was more effective than germination percentage (Fig. 1 and Fig. 4).

**Discussion**

The abnormalities of mitosis directly affected the cell division and growth rate, which showed marked symptoms of Cd toxicity, such as the inhibition of root elongation and morphological change. The Cd concentration that caused significant inhibition for shoot growth was much higher than that for root growth. Although Cd had no significant effects on seed germination, radicle and shoot growth were markedly inhibited by Cd (He *et al.,* 2008). In other words, shoot growth proved to be more sensitive to cadmium stress. The reduction in growth could be a consequence of the Cd interference with a number of metabolic processes associated with normal development such as photosynthetic pigments production, membrane lipid composition, water uptake and mineral nutrition that would result in deficiency in essential elements and ultimately reduction in biomass production (Ammar *et al.,* 2008).

Seed priming is a pre-sowing strategy for influencing seed germination and seedlings' development by modulating pre-germination metabolic activity prior to emergence of the radicle and generally enhancing germination rate and plant performance (Bradford, 1986). According to the current results, most of cadmium's toxic effects were observed on shoot length reduction as this parameter was more affected by priming treatments than the other parameters, so that salicylic acid, potassium nitrate and potassium chloride increased the shoot length with 214.16%, 100% and 109.16%, respectively. However, these values were lower in the other parameters.

All treatments compared to the control resulted in a higher germination rate. These findings are in agreement with the results reported by (Demir and Mavi, 2004) who proved that salt priming can increase watermelon emergence. Farooq *et al.* (2007) noticed that halopriming with KCl improved germination and emergence in rice. Also, Farooq *et al.* (2006) reported that seed priming with KCl or $CaCl_2$ improved the rate of germination, the length of shoot and root in rice seedling. Treated seeds of two celery varieties with $KNO_3$ and KCl showed that the germination rate has increased, compared to the control (Perez-Garcia *et al.,* 1995); the highest germination rate was recorded in $KNO_3$ and SA treatments. Amjad *et al.* (2007) reported $KNO_3$ to be better than other treatments by decreasing germination time to 50%. It is plausible that $KNO_3$ positive effect might be due to its role in influencing the permeability of the membranes, which ultimately leads to activation of enzymes involved in protein synthesis and carbohydrate metabolism (Preece and Read, 1993). Moreover, it plays role in formation of protoplasm and new cells, as well as encourages plant elongation. Also, potassium is a major essential element required for physiological mechanisms of plant growth (Aisha *et al.,* 2007). Increase in shoot and radicle length might be due to induction of metabolic activates in embryo as a result of seed priming (Wahid *et al.,* 2008).

However, some results show that the decrease of germination index in seeds primed with $KNO_3$ at high levels might be due to toxic effects of solution on the embryo (Giri and Schillinger, 2003). According to Frett *et al.* (1991) using inorganic salts for the preparation of osmotic solution, due to osmotic damage to cell membranes and changes of enzyme that effect on germination, seeds can be harmed. The hereby study testing did not show such effects.

The application of -0.3 MPa of $KNO_3$ increased the germination index compared to control. The experiment of Ramzan *et al.* (2010) have also led to an increase in the concentration of potassium nitrate that resulted in reduced germination percentage of gladiola plants, while at low concentrations germination was improved; this may be because high salt concentrations caused enhancement of cell death and reduced improvement of germination indices (Nascimento, 2003). However, greater efficiency of osmohardening with KCl is possibly related to the osmotic advantage that both $K^+$ and $Ca^{2+}$ have in improving cell water saturation, and that they act as co-factors in the activities of numerous enzymes.

Salicylic acid could form a complex with Cd that may provide Cd tolerance (Choudhury and Panda, 2004). One of the important roles of SA in inducing resistance to various environmental stresses is manifested by its ability to express genes that code for pathogenesis-related proteins or defense-related enzymes (Merkouropoulos *et al*., 1999). The SA pretreatment alleviates Cd toxicity in barley (Metwally *et al*., 2003) and maize plants (Krantev *et al*., 2008). Significant decline in root length and dry weight was observed in SA free roots. The SA priming resulted in the increase of root length and dry weight. Reducing the effects of stress is explained by the fact that salicylic acid enhances some of plant hormones, such as auxin and cytokinin (Shakirova *et al.,* 2003), while reducing ion leakage from the plant cells and decreasing accumulation of toxic ions in plants (Borsani *et al.,* 2001). Salicylic acid through the development of anti-stress reactions such as the accumulation of proline, improves the growth of seedlings after the elimination of stress (Shakirova *et al.,* 2003). Salicylic acid enhances germination parameters because of the protective action in the presence of heavy metals, leading to the stability of cell membrane (Mishra and Choudhuri, 1999). Changes in hormone balance (Shakirova *et al.,* 2003) and cadmium ions become inactive (Metwally *et al.,* 2003), aiming for removal of this metal from metabolic processes and reduction of its toxicity in spite of increasing concentrations. There are reports showing that seed priming permits early DNA replication, increases RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites (McDonald, 2000). This technique helped seedlings grow in stress conditions (Ashraf and Foolad, 2005).

## Conclusions

After long-term exposure to cadmium, roots became mucilaginous, browning and decomposing; reduction of shoots and root elongation can occur as well. Cd was found to inhibit lateral root formation, while the main fenugreek root became brown, rigid and twisted. In 30 mg/L of cadmium concentration, shoot growth decreased more than root growth and salicylic acid, potassium nitrate and potassium chloride increased the shoot length with 214.2, 100 and 109.2%, respectively compared to the control. This indicated that shoot length was strongly influenced by the treatments and increased more than other germination parameters, while salicylic acid had the most protective effect. Highest germination indices were observed in seeds primed with SA under various stress levels, because SA could form a complex with cadmium that may provide Cd tolerance. Because the protective action of SA in the presence of heavy metals which led to the stability of cell membrane, changes in hormone balance and inactivity of cadmium ions could be observed. Even though $KNO_3$ and KCl treatments may also cause enhancement of germination indices, since the treatment with salicylic acid showed better results for all germination indices, it can be concluded that salicylic acid is the best seed priming agent to reduce the toxicity of cadmium in fields exposed to Cd-polluted water.

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