



## Seed germination of dill (*Anethum graveolens* L.) in response to salicylic acid and halopriming under cadmium stress

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### Abstract

Seed priming is a technique which is potentially able to promote rapid and more uniform seed germination and plant growth as well. In this regards, the presowing effects of seed priming treatments were investigated on some physiological viz. seed germination, germination rate, radicle elongation, shoot elongation, radicle dry weight, shoot dry weight and vigor index. Seeds were subjected to two seed priming methods, hormopriming, and halopriming. Also, untreated seeds were used as control. Halopriming with -0.5 MPa of KNO<sub>3</sub> and KCl and hormopriming with 100 mg/L of SA at 15 °C were used for 24 h in a factorial experiment with completely randomized design under laboratory conditions. The results showed that hormopriming (100 mg/L of SA) improved the germination rate (no significant difference with KNO<sub>3</sub> and KCl), germination percentage, radicle elongation, plumule elongation, radicle dry weight, plumule dry weight, and vigor index under cadmium stress (30 mg/L) at 56.35, 11.9, 30.65, 30.33, 65.21, 71.42, and 46.16%, respectively. In all cadmium concentrations, the maximum of all germination parameters were related to the seeds primed with SA solution. While in primed seed by KNO<sub>3</sub> and KCl treatments, exposure to 20 and 30 mg/L Cd decreased radicle length as compared to the control. Also, exposure to 30 mg/L Cd, KNO<sub>3</sub> solution showed lower plumule length than control. The amount of reduction in radicle length in primed seeds with KNO<sub>3</sub> solution and KCl solution was 11.88% and 6.18%, respectively, in 30 mg/L Cd. However, in all cadmium concentrations, germination percentage and rate were enhanced by KNO<sub>3</sub> and KCl solubles.

**Key words:** Heavy metal, radicle length, seed priming, medicinal plant

**Abbreviations:** Cadmium (Cd), Salicylic acid (SA), Potassium nitrate (KNO<sub>3</sub>), Potassium chloride (KCl)

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### Introduction

Cadmium is a non-essential, toxic and dangerous pollutant (Ekvall, and Greger, 2003) and a common transitional metal and available in the environment (Morsy et al., 2011) and its biodegradation does not easily occur (Ikem and

Egibor, 2005). Water pollution with cadmium represents an important environmental problem. Cadmium has high priority for removal from aqueous solutions (Rama et al., 2002). Cadmium alters the morphological, physiological, biochemical, and structural processes in plants (Mishra et al., 2006), which is resulting from alterations of numerous physiological processes caused at cellular/molecular level by inactivating

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enzymes, blocking functional groups of metabolically important molecules, displacing or substituting for essential elements, and disrupting membrane integrity. A rather common consequence of cadmium poisoning is the enhanced production of reactive oxygen species (ROS) due to interference with electron transport activities, especially that of chloroplast membranes (La Rocca et al., 2009). This increase in ROS exposes cells to oxidative stress leading to lipid peroxidation, biological macromolecule deterioration, membrane dismantling, and ion leakage (Quartacci et al., 2001) which causes reduction in water content (Siswanto et al., 2013).

Toxic effects of cadmium on morphological processes cause inhibition and abnormalities of general growth in many plant species. After long-term exposure to Cd, roots are mucilaginous, browning, and decomposing; Moreover, reduction of plumules and root elongation, rolling of leaves, and chlorosis can occur. 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 is disordered division and abnormal enlargement of epiderma and cortical cell layers in the apical region (Miyadate et al., 2011).

Germination and seedling emergence is the most important and critical stage of plant growth because at this stage, seeds are in unfavorable environmental circumstances (Bandara and Slinkard, 2000). Roots are the first area facing cadmium so the reaction between cadmium and root cells lead to changes in the physiological properties of cells plasma membranes (Astolfi et al., 2003). Inhibition of seed germination and growth by cadmium has been reported by many researchers (Shafiq and Iqbal, 2005).

Dill (*Anethum graveolens* L.) is an annual herb of the Apiaceae family. It is native to southwestern Asia and southeastern Europe. It has been cultivated since ancient times as a vegetable, a carminative, an aromatic, and an antispasmodic plant. It is considered to be one of the most important medicinal plants in Iran, after saffron (*Crocus sativus* L.), cumin (*Cuminum cyminum* L.), and fennel (*Foeniculum vulgare* Mill.) (Mirshekari, 2012). This plant has a small seed,

which may be unequal germination in stress conditions. In stress conditions, priming is action by which the seeds are partially hydrated and then dried to get initial moisture in water (hydration) or in a solution of organic or mineral salts (Osmopriming). Such treatment improves and accelerates germination and subsequent growth of the seedling (Farooq et al., 2006).

Ghassemi-Golezani et al (2010) evaluated the positive effects of osmo-priming (KNO<sub>3</sub> and NaCl) on seed germination. Seedling growth were reported for barley, cucumber, fennel (*Foeniculum vulgare*) and winter rapeseed (*Brassica napus*) and it was concluded that salt priming, particularly KNO<sub>3</sub> priming, decreased mean germination time and increased seedling size, compared with non-primed seeds (Ghassemi-Golezani et al., 2010).

Seed priming with potassium nitrate significantly increased germination rate and seedling dry weight in dill plant under normal condition. It might be due to early synthesis of nucleic acids, DNA, RNA, and proteins during salt hydration process, which ultimately resulted in improved energy of germination of seeds (Ghassemi-Golezani et al., 2011).

Salicylic acid belongs to a group of phenolic compounds that as an important molecule is known to modulate plant responses to environmental stresses (Senaratna et al., 2000). Cadmium content in SA free roots was higher as compared to that in SA primed roots (Choudhury and Panda, 2004). The SA growth inducing properties are reported in barley (Metwally et al., 2003) and rice (Choudhury and Panda, 2004) under Cd treatment. In addition, it was demonstrated that SA pretreatment decreased MDA accumulation caused by Cd, which confirm the role of this compound against oxidation damage (Choudhury and Panda, 2004).

The trend of water sources pollution of semi-arid areas to heavy metals such as cadmium is increasing (Rama et al., 2002). Furthermore, scarce of available water resources has led to the use of contaminated water for irrigation of cropping systems. In the farming systems, dill plant is cultivated in irrigated farming exposed to heavy metal stress. Since the germination is the most sensitive stage to heavy metal stress, the present study aimed to determine the effect of seed priming treatments in reducing the toxic

effects of cadmium on seed germination of plants sensitive to cadmium like dill seed.

**Material and Methods**

**Seed priming**

The dill seeds (*Anethum graveolens* L.) were purchased from Pakan Bazr, Isfahan, Iran. Then, the factorial experiment was conducted in completely randomized design with four replications at faculty of Agriculture, Shahrekoord University, Iran, in 2014. Seeds were rinsed with distilled water and then surface sterilized in 40% sodium hypochlorite (vol/vol) for 10 minutes, followed by 70% alcohol (vol/vol) for 10 second before use when they were thoroughly rinsed with sterile deionized water and then were primed.

Treatments were different halopriming and hormopriming levels. For halopriming dill seeds were placed on potassium nitrate (1 MPa KNO<sub>3</sub>) and potassium chloride (1 MPa KCl). For hormopriming, dill seeds were placed on a salicylic acid (SA) solution at 100 mg/L of salicylic acid in the chamber room, under dark condition at 15 °C for 24 h, then rinsed with distilled deionized water (dd H<sub>2</sub>O three times to wash off the KNO<sub>3</sub>, KCl and SA solutions from the surface of the seeds and air dried for 24 h at 25 °C to reach the original moisture content (~12–13%) and immediately were used for germination tests. Unprimed seeds were used as control.

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

$$\phi_s = -miRT$$

- $\phi_s$  = Osmotic potential (MPa)
- m = Molality of solution (M dissolved in 1000 g of water)
- i = Ion coefficient (equal to 2 for salts dissolved in water)
- R = Fixed amount of gas (00831/0 L MPa per M K)
- T = Temperature in degrees Kelvin = temperature in degrees Celsius + 273

**Germination of primed and unprimed seeds under different cadmium concentrations**

For germination test, unprimed seeds were surface sterilized like primed seeds. A total

of 200 seeds (primed and unprimed seeds) 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 Cd Cl<sub>2</sub>, at 0, 10, 20 and 30 mg/L levels of Cd Cl<sub>2</sub>, constituted an experimental unit for all experiments, and were incubated in a seed germinator, under dark condition at alternating 20-30 °C temperature. Seeds were considered germinated when at least 2 mm long radicle protruded through the seed coat (ISTA, 2009).

Germination percentage (GP), germination rate, radicle and plumule length, plumule dry weight (DSW), radicle dry weight (DRW), and vigor index (VI) were recorded to evaluate germination performance after 22 days. Daily germination percentage was recorded and subjected to statistical analysis. The root and plumule vigor were calculated as the sum of total root length (cm) and plumule lengths (cm) of all seedlings of a replicate were divided by 20. Fresh radicle and plumule were measured and then placed in a hot air oven (70 °C for 24 h) to dry (ISTA, 2009). Radicle and plumule dry mass was measured with electrical scale.

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

$$GP = \frac{\text{Germinated seeds after 14 day}}{\text{Total planted seeds}} \times 100 \quad (\text{Equation I})$$

Germination rate (T50) was defined as days needed to reach 50% of GP. Coefficient of velocity of germination (CVG) was calculated using Equation II (Kotowski, 1926):

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

Where 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 plumule length by germination percentage (Abdul-Baki and Anderson, 1973).

Seed vigor index was calculated using Equation III (Kalsa and Abebie, 2012):

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

## Data Analysis

Data was analyzed statistically using analysis of variance in SAS 9.1 and the LSD test ( $p < 0.01$ ) to determine whether differences among means were significant between treatments within haloprimering, hormoprimering, and cadmium stress.

## Results

Analysis of variance showed that seed priming, cadmium concentration, and their interactions had significant effect on germination rate and percentage, radicle length and plumage length (Table I) and radicle dry weight, plumule dry weight and vigor index (Table II).

### Germination percentage

Total germination from both primed and non-primed seeds decreased significantly with increasing cadmium concentration (data not shown). However, this reduction in total germination was significantly higher for non-primed seeds, compared to primed seeds. Data suggested a reduction of 4.54% in non-primed seed on total germination due to an increase in cadmium concentration from 0 mg/L to 30 mg/L (Fig. I). Results indicated that seed priming increase germination by 12, 8 and 6.34% using SA seed priming,  $\text{KNO}_3$  seed priming and KCl seed priming, respectively, compared to non-primed seeds. In fact, exposed to 30 mg/L of cadmium, seed primed with SA showed the highest germination percentage (70.5%) while control seeds showed the lowest total germination (63%). In 0.5 MPa  $\text{KNO}_3$  and KCl pretreatment, seed germination reached 67% and 68%, respectively (Fig. I).

Seed priming by  $\text{KNO}_3$  did not significantly increase germination percentage ( $P > 0.01$ ) in comparison with control seeds, when exposed to 0 and 20 mg/L of cadmium stress (Fig. I). Exposure to 10 and 30 mg/L of cadmium,  $\text{KNO}_3$  pretreatment showed higher level than the KCl pretreatment, while in 20 mg/L of Cd,  $\text{KNO}_3$  pretreatment was not significantly different from KCl pretreatment (Fig. I).

### Germination rate

Statistical analysis regarding days taken to 50% germination is presented in Table I. The difference between primed and non-primed seeds for days taken to 50% germination was also significant. Germination rate decreased with increasing cadmium concentration. The non-primed seed took significantly more days to 50% germination as compared to primed seed and this indicates that germination rate was slowly in non-primed seed compared to primed seed. The highest germination rate was recorded in seeds primed with SA solution at all cadmium concentrations, except in 20 mg/L where  $\text{KNO}_3$  solution showed higher germination rate than other treatments and was not significantly different from  $\text{KNO}_3$  and KCl solution in 30 mg/L Cd. Exposure to 0 and 20 mg/L Cd, KCl solution was better than  $\text{KNO}_3$  while in 10 mg/L Cd,  $\text{KNO}_3$  solution was better than KCl solution. At all cadmium concentrations, the lowest germination rate was recorded in non-primed seed. SA,  $\text{KNO}_3$ , and KCl solutions increased germination rate by 56.35%, 52.11%, and 55.88%, respectively (Fig. II).

### Plumule length

The results of analysis of variance indicated that priming and cadmium concentration and their interaction significantly affected plumule length (Table I). There was a significant difference between plumule length of primed and non-primed seeds. Plumule length from both primed and non-primed seeds decreased significantly with increasing cadmium concentration. However, this reduction in plumule length was significantly higher for  $\text{KNO}_3$  solution (40.40%) and KCl solution (36%) compared to non-primed seeds (33.61) and SA solution (22.35%) in cadmium concentration from 0 mg/L to 30 mg/L.

Primed seed attained more length than non-primed seeds. Exposure to 30 mg/L Cd, plumule length increased with SA solution and KCl solution by 30.33% and 1.02%, respectively while  $\text{KNO}_3$  solution decreased this parameter by 2.57%. Exposure to 0 and 10 mg/L Cd,  $\text{KNO}_3$ , and KCl treatments had significantly positive effects on plumule length when compared to the control. While in concentration of 20 and 30 mg/L Cd, these treatments were not significantly different from control. Exposure to 30 mg/L Cd, primed

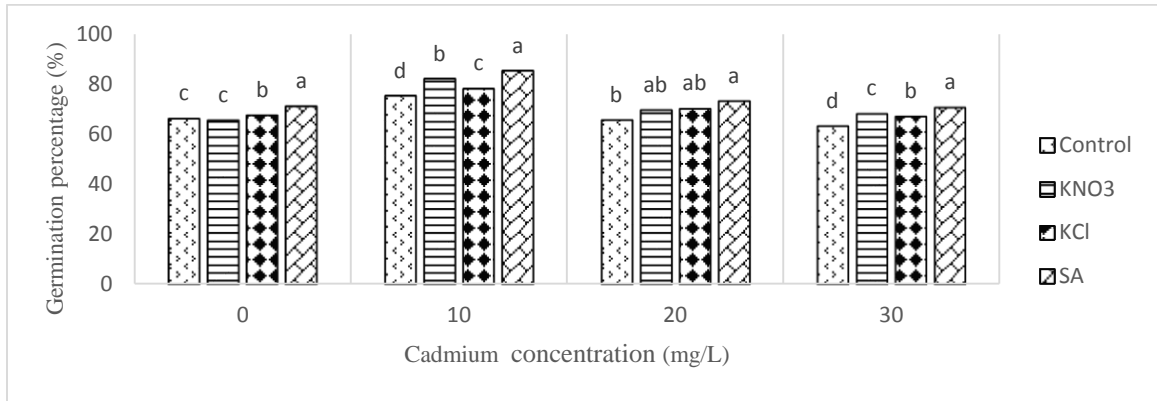


Fig. I. Effect of priming treatments on the germination percentage under different cadmium concentrations. Means with similar letters are not significant different ( $p \leq 0.05$ ) based on LSD test

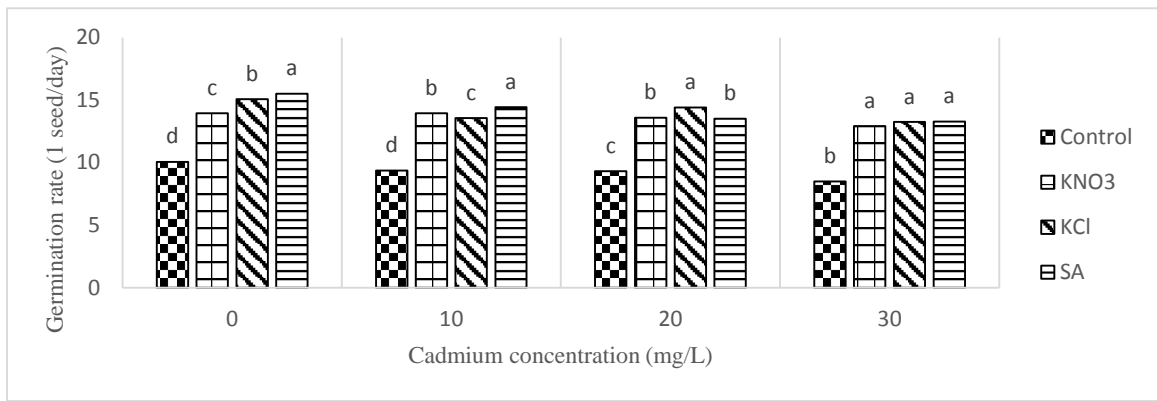


Fig. II. Effect of priming treatments on the germination rate under different cadmium concentrations. Means with similar letters are not significant different ( $p \leq 0.05$ ) based on LSD test.

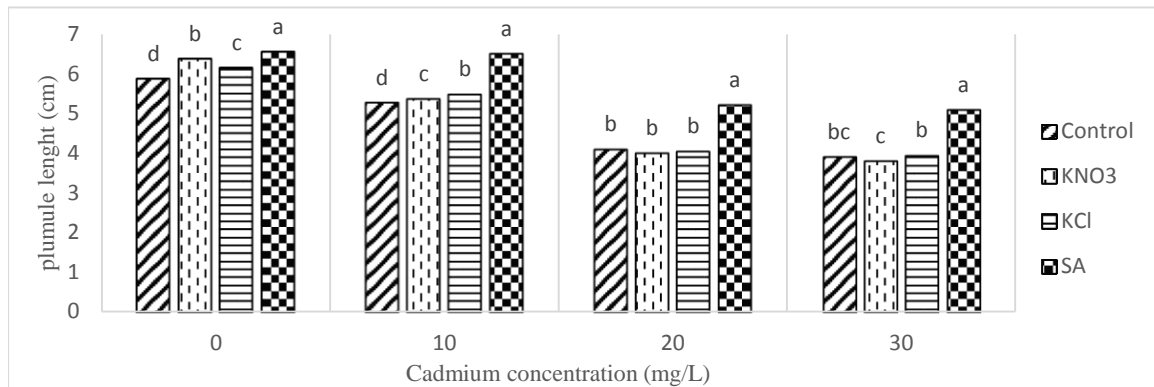


Fig. III. Effect of priming treatments on the plumule length under different cadmium concentrations. Means with similar letters are not significant different ( $p \leq 0.05$ ) based on LSD test

seeds with SA treatment resulted in highest plumule length while lowest plumule length was recorded at KNO<sub>3</sub> treatment (Fig. III).

### Radicle length

There was a significant interaction effect of seed treatment × cadmium concentration for radicle length (Table I). Cadmium had a significant inhibitory effect on radicle length for both primed and non-primed seeds. However, this effect was significantly ( $P < 0.01$ ) less pronounced in seedlings from SA seed priming, KNO<sub>3</sub> seed priming, and KCl seed priming in comparison with control seeds (Fig. IV). However, KNO<sub>3</sub>, and KCl treatments did not give positive response in comparison with SA treatment when exposed to 20 and 30 mg/L of cadmium. After exposure to 30 mg/L Cd, the amount of reduction in primed seeds with KNO<sub>3</sub> solution and KCl solution was 11.88% and 6.18%, respectively. Exposure to 0 and 10 mg/L Cd, KNO<sub>3</sub>, and KCl treatments showed greater radicle length than the control.

The maximum and minimum radicle length has been recorded in seeds primed with SA treatment and control, respectively in all concentrations of cadmium. SA priming resulted in increase in radicle length. Under SA primed

conditions, the increase was significantly further in comparison to SA free radicle, which showed 30.69% increase at 30 mg/L of cadmium. The radicles of dill seedlings were more sensitive to Cd than plumule of dill seedlings and reduced to a greater extent. (Fig. IV).

### Plumule dry weight

Plumule dry weight was also significantly affected by cadmium stress, priming, and interaction of priming and cadmium stress (Table II). Plumule dry weight was found to decrease significantly with increase in cadmium concentration.

Table (II) exhibits the data pertaining to plumule dry weight of dill seeds. The difference between primed and non-primed seed for Plumule dry weight was statistically significant. Increasing cadmium significantly ( $P < 0.01$ ) decreased plumule dry weight for both primed and un-primed seeds. However, primed seeds showed a better performance than non-primed seeds, except for KNO<sub>3</sub> treatment that showed lower plumule dry weight than control at 30 mg/L Cd. Data in (Fig. V) show that SA seed priming enhanced dill seedling dry weight to about 71.42% as compared to control. Also the highest plumule dry weight was

Table 1

Analysis of variance (mean square) for effects of seed priming on germination rate, germination percentage, root length and radicle length of dill under different cadmium concentrations.

Source of variance	df	Germination percentage	Germination rate	Plumule length	Radicle length
Cadmium (Cd)	3	140.56**	4.75**	16.40**	22.19**
Seed priming (P)	3	132.72**	337.72**	3.78**	8.64**
Cd × P	9	15.34**	2.08**	0.19**	0.95**
Error	48	0.85	1.19	0.003	0.0027
C.V (%)		1.15	3.58	1.12	2.12

\*\* Significant at 0.01 probability level.

Table 2

Analysis of variance (mean square) for effects of seed priming on, radicle dry weight, plumule dry weight and vigor index of dill under different cadmium concentrations.

Source of variance	df	Plumule dry weight	Radicle dry weight	Vigor index
Cadmium (Cd)	3	0.000011**	0.0000076**	194593.8**
Seed priming (P)	3	0.000012**	0.000011**	90341.61**
P × Cd	9	0.0000002**	0.00000028**	8496.48**
Error	48	0.00000001	0.00000003	42.95
C.V (%)		2.38	5.38	1.46

\*\* Significant at 0.01 probability level.

observed in seedlings pretreated with SA solution while the lowest plumule dry weight was obtained from seedlings pretreated with KNO<sub>3</sub> solution (Fig. V). The amount of reduction in primed seeds with KNO<sub>3</sub> solution was 7.14%. Exposure to 0 and 10 mg/L Cd, KNO<sub>3</sub>, and KCl solutions showed the highest plumule dry weight as compared to control; however, in 20 mg/L Cd, no significant difference was found between control group, KNO<sub>3</sub>, and KCl treatments. In all cadmium

concentrations, KCl treatment showed higher plumule dry weight as compared to KNO<sub>3</sub> treatment, except in 20 mg/L Cd, that made no significant difference between groups (Fig. V).

**Radicle dry weight**

Radicle dry weight was significantly affected by seed priming treatment ( $p < 0.01$ ). Interaction of priming treatment  $\times$  cadmium

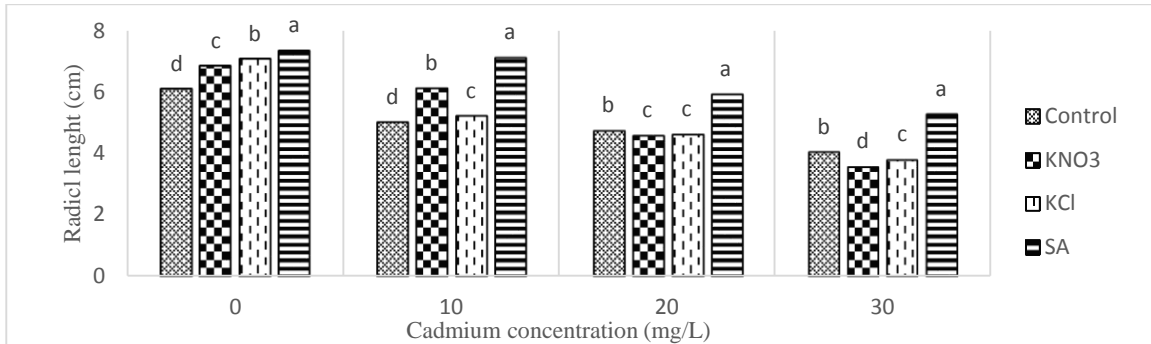


Fig. IV. Effect of priming treatments on the radicle length under different cadmium concentrations. Means with similar letters are not significant different ( $p \leq 0.05$ ) based on LSD test.

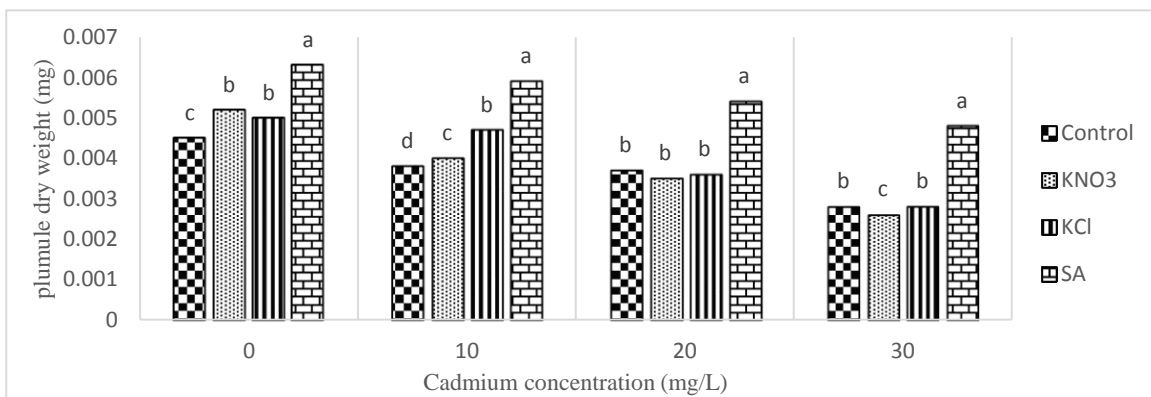


Fig. V. Effect of priming treatments on the Plumule dry weight under different cadmium concentrations. Means with similar letters are not significant different ( $p \leq 0.05$ ) based on LSD test

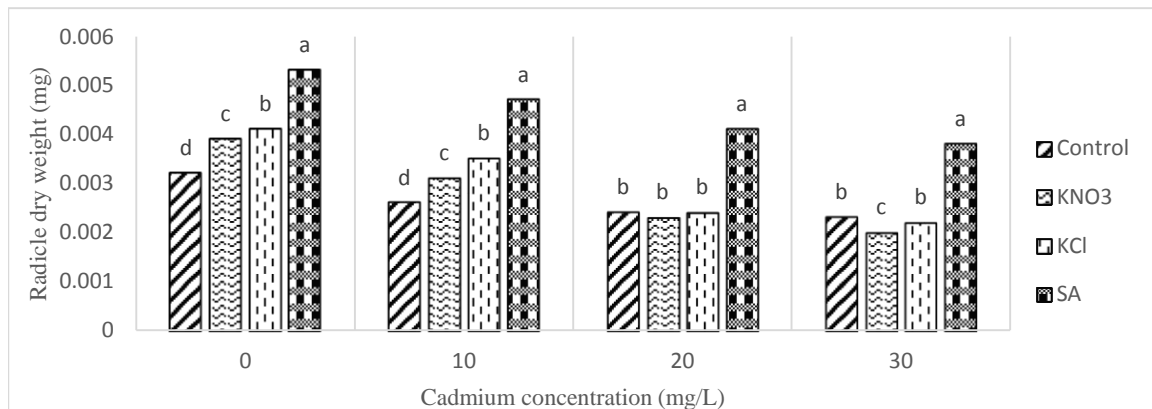


Fig. VI. Effect of priming treatments on the radicle dry weight under different cadmium concentrations. Means with similar letters are not significant different ( $p \leq 0.05$ ) based on LSD test

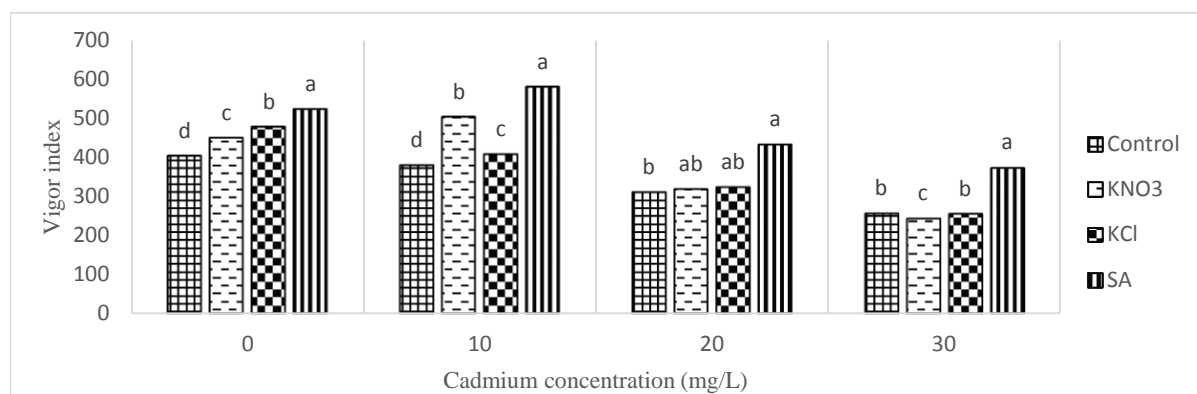


Fig.

VII. Effect of priming treatments on the Radicle dry weight under different cadmium concentrations. Means with similar

concentration had significant effect on radicle dry weight (Table II). Radicle dry weight significantly decreased with the increase in levels of cadmium as compared to the control.

Seed pretreatments significantly affected radicle dry weight at different cadmium levels. Hormopriming technique with SA treatment compared with halopriming with KNO<sub>3</sub>, and KCl treatments clearly improved radicle dry weight under both stress and non-stress conditions.

In the 30 mg/L CdCl<sub>2</sub> treatment, an increase of approximately 65.21% was detected in the seeds primed with SA solution as compared with the control group ( $P < 0.01$ ). Interestingly, SA not only was able to prevent reduction in radicle dry weight, but also improved the radicle dry-weight of seedlings exposed to Cd, compared to other treatments.

Radicle dry weight was decreased as a result of pretreatment with KNO<sub>3</sub> solution (13%), and the reduction was significant and it was enhanced by pretreatment with SA and KCl solution 65.21% and 4.34%, respectively as compared to the control. Exposure to 0 and 10 mg/L Cd, KNO<sub>3</sub> and KCl solutions showed the most plumule dry weight as compared to control; however, 20 mg/L Cd did not make a significant difference between control group, KNO<sub>3</sub>, and KCl treatments (Fig. VI).

### Vigor index

Analysis of variance indicated that both cadmium level and priming have significant effects on vigor index. Furthermore, cadmium level  $\times$  priming interaction was significant according to

LSD test at  $P < 0.01$  level (Table II). Increasing cadmium concentration causes a significant decrease ( $P < 0.01$ ) in dill vigor index for both control and primed seeds (data not shown). The amount of reduction in seeds primed with SA solution, KNO<sub>3</sub> solution, and KCl solution was 28.8, 46, and 46.78%, respectively and in the control this was 36.76%. The highest amount of reduction was found in seeds primed with KCl solution.

SA priming increased the vigor index in all cadmium concentrations and made significant difference with other treatments, except in 20 mg/L cadmium that did not show significant difference with KNO<sub>3</sub> and KCl treatments. Exposure to 30 mg/L cadmium, SA seed priming has significantly ( $P < 0.01$ ) increased vigor index to about 46.16%, while KNO<sub>3</sub> and KCl seed priming decreased vigor index to about 4.88 and 0.3%, respectively in comparison with control seeds (Table II). However, exposure to 0 and 10 mg/L Cd, KNO<sub>3</sub>, and KCl treatments had a better result than the control (Fig. VII).

### Discussion

Application of different levels of CdCl<sub>2</sub> in dill plants adversely decreased their growth pattern (germination percentage and rate, radicle and plumule length, radicle and plumule dry weight, and vigor index) as compared with control plants. These results are in agreement with those of Lopez-Millan et al. (2009) in tomato who showed that cadmium caused a significant reduction in growth parameters. Cadmium growth inhibition could also be due to the inhibition of cell division and elongation rate of cells that results in



a decline in biomass production. This result mainly occurs by an irreversible inhibition of proton pump responsible for the process (Choudhury and Panda, 2004).

Cd induced mineral stress could reduce seedling dry weight accumulation (Marshner, 2012). Dry weight decrease in the presence of Cd was also confirmed by Cailin et al. (2009) and Kummerová et al. (2010) for *Triticum aestivum* and *Matricaria recutita*, respectively.

SA growth inducing properties are reported in barley roots under Cd treatment (Matewally et al., 2003). Lines of evidence are indicative that endogenous SA may function as a signaling molecule in response to Cd-induced oxidative stress (Panda and Patra, 2007). Consequently, one may foresee that exogenous application of SA has ameliorative effects on plants experiencing Cd toxicity. There are many studies on this subject. Short-term treatments of SA were found to have more positive results on the plant growth, photosynthesis, and antioxidant system (Krantev et al., 2006).

The beneficial effect of SA was seen on all growth parameters in dill. The same positive effect of SA on growth was reported by Metwally et al. (2003) in the presence of Cd exposed to cadmium, reduced root and plumule length and fresh weight in barley seedlings and SA treatment decreased Cd toxicity. These results in response to Cd stress and SA are also in agreement with those of Popova et al. (2008) in pea plant and Shi et al. (2009) in hemp plants. SA is needed for the adaptation process and the induction of stress tolerance (Popova et al., 2008).

It is assumed that the beneficial effects of SA during a growth period can be related to avoidance of cumulative damage upon exposure to cadmium or modification of compartmentalization. Alternatively, SA could be involved in the expression of specific proteins or defense-related enzymes (Krantev et al., 2006). SA can also form a complex with Cd that may provide Cd tolerance (Moussa and EL-Gamal, 2010). The effects of stress are mitigated because salicylic acid enhances some plant hormones such as auxin and cytokinin (Sharikova et al., 2003), reduces ion leakage from the plant cells, and decreases accumulation of toxic ions in plants (Borsani et al., 2001). Salicylic acid enhances germination

parameters because its protective action in the presence of heavy metals leads to the stability of the cell membrane (Mishra and Choudhuri, 1999), changes in hormone balance (Sharikova et al., 2003), and inactivity of cadmium ions (Metwally et al., 2003), removal this metal from metabolic processes and reduce the toxicity of cadmium in spite of increasing concentrations.

KNO<sub>3</sub> enhanced germination rate and percentage even at highest cadmium concentration. It is plausible that the positive effect of KNO<sub>3</sub> might be because of 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). Also germination enhancement in seed primed with KNO<sub>3</sub> may be attributed to metabolic repair processes, a buildup of germination metabolites or osmotic adjustments during priming treatments (Bray et al., 1989).

KNO<sub>3</sub> and KCl treatments in initial concentration of cadmium (0 and 10 mg/L) increased radicle and plumule length. It may be due to availability of nitrogen and potassium from priming solution. The enhanced seedling length in primed seeds may be because of the improved and faster plants seedling emergence and plant length may be because of the efficiency of the plant for utilization of nitrogen which is essential for plant growth as well as other processes related to nitrogen metabolism (El-Bassiony, 2006).

Increase in plumule and radicle length might be due to induction of metabolic activities in embryo as a result of seed priming (Wahid et al., 2008). At water imbibition during germination process, DNA cloning, RNA stimulated activity and consequently protein synthesis as well as hormone concentration in the primed seeds are increased improving germination and seedling growth (Ashraf and Foolad, 2005). Seed priming leads to the initiation of primary metabolic processes, so the time required for germination is reduced. This positive effect is probably due to the stimulatory effect of priming on later stages of the germination process through the mediation of cell division in germinated seeds (Sivritepe et al., 2003).

The findings of Ruan et al. (2002a) demonstrated that priming the rice seed with KCl and CaCl<sub>2</sub> had improved results for germination index. Greater efficiency of seed priming with KCl is possibly related to the osmotic advantage that K<sup>+</sup> has in improving cell water saturation, and that they act as co-factors in the activities of numerous enzymes (Taiz and Zeiger, 2002). Farooq et al. (2007) noticed that halopriming with KCl improved germination and emergence in rice.

Some results show the decrease index germinations in seeds primed with KNO<sub>3</sub> at high levels probably is due to the toxic effects of solution on the embryo of seed (Giri and Schillinger, 2003). According to Frett et al. (1991) using inorganic salts for the preparation of osmotic solution for seeds can be harmful due to osmotic damage to cell membranes and changes of enzyme that affect germination. Toxic ion accumulation negatively affects plant metabolism (Grieve and Fujiyama, 1987).

It was found that priming with KNO<sub>3</sub> greatly influences the plumule length as nitrogen supplied by KNO<sub>3</sub> is an indispensable elementary constituent of numerous organic compounds such as amino acids, proteins and nucleic acids. Moreover, it plays a role in formation of protoplasm and new cells as well as encourages plant elongation. Also, potassium is major essential element required for physiological mechanism of seedling growth (Aisha et al., 2007).

Although some earlier studies reported that halopriming can contribute to improved germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao et al., 1999), enhancement of ATPase activity, RNA, and acid phosphatase synthesis (Fu et al., 1988), and also by increase of amylases, protease or lipases activity (Ashraf and Foolad, 2005).

## Conclusion

Trend of water sources pollution in increasing to heavy metals such as cadmium that have spread through human activities and is transferred rapidly in soil and water. The application of this polluted water in the farming systems alters the morphological, physiological, biochemical, and structural features of the plants

including inhibition of germination and growth of seedling. After long-term exposure to Cd, roots become mucilaginous, brown, and decomposing; reduction of plumule and root elongation can also occur. Cadmium was found to inhibit lateral root formation while the main root became brown, rigid, and twisted. This effects was showed in dill plants. The main reason is disordered division and abnormal enlargement of epiderma and cortical cell layers in the apical region. In 30 mg/L of cadmium, radicle growth decreased more than plumule growth and salicylic acid increased the radicle length to 30.69% while potassium nitrate and potassium chloride decreased radicle length to 11.88% and 6.18%, respectively compared with the control. This indicates the plumule length was strongly influenced by treatments and increased more than other germination parameters. In fact, salicylic acid had the most protective effect. Exposed to 30 mg/L Cd, plumule length increased with SA and KCl solution by 30.33, and 1.02%, respectively while KNO<sub>3</sub> solution decreased this parameter by 2.57%. The lowest radicle length was recorded in seed primed with KNO<sub>3</sub> treatment and 30 mg/L Cd. However, in all cadmium concentrations, germination percentage and rate were enhanced by KNO<sub>3</sub> and KCl solutions. The amounts of improvement in germination percentage in seeds primed with KNO<sub>3</sub> and KCl solution were 8%, and 6.34%, respectively and for germination rate they were 52.11%, and 55.88%. The highest germination indices were observed in seeds primed with SA treatment under various stress levels because SA could form a complex with Cd that may provide Cd tolerance. The lowest germination indices on the other hand, were observed in no primed seeds under 30 mg/L of cadmium. SA treatment in all cadmium concentrations was known as appropriate because it could form a complex with Cd that may provide Cd tolerance for dill germination parameters. Consequently, the use of salicylic acid in fields irrigated with contaminated water is recommended, to reduce toxicity and create better conditions for growth.

## References

- Abdul-Baki, A.A. and J.D. Anderson.** 1973. 'Vigor determination in soybean seed by multiple criteria'. *Crop Science*, 13:630–633.

- Aisha, A.H., F.A. Rizk, A.M. Shaheen and M.M. Abdel-Mouty.** 2007. 'Onion plant growth, bulb yield and its physical and chemical properties as affected by organic and natural fertilization'. *Research Journal of Agriculture and Biological Sciences*, 3(5):380-388.
- Ashraf, M. and M.R. Foolad.** 2005. 'Pre-sowing seed treatment-a shotgun approach to improve germination growth and crop yield under saline and none-saline conditions'. *Advan. Agronomy*, 88:223-271.
- Astolfi, S., S. Zuchi, C. Chiani and C. Passera.** 2003. 'In vivo and in vitro effects of cadmium on H+ATPase activity of plasma membrane vesicles from oat (*Avena sativa* L.) roots'. *Journal of Plant Physiology*, 160 (4):387-393.
- Bandara, M. and A.E. Slinkard.** 2000. 'Seed priming improves stand establishment in Anise (*Pimpinella anisum*)'. *Kernels*, 2: 12-16.
- Borsani, O., V. Valpuesta and M.A. Botella.** 2001. 'Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings'. *Plant Physiology*, 126:1024-1030.
- Bray, C.M., P.A. Davison, M. Ashraf and R.M. Taylor.** 1989. 'Biochemical changes during osmopriming of leek seeds'. *Annals of Botany*, 63:185-193.
- Cailin, G.E., Y. Ding, Z. Wang, D. Wan, Y. Wang, Q. Shang and S. Luo.** 2009. 'Responses of wheat seedlings to cadmium, mercury and trichlorobenzene stresses'. *Journal of Environmental Sciences*, 21:806-813.
- Choudhury, S. and S.K. Panda.** 2004. 'Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots'. *Bulg. Journal of Plant Physiology*, 30:95-110.
- Ekvall, L. and M. Greger.** 2003. 'Effects of environmental biomass-production factors on Cd uptake in two Swedish ecotypes of *Pinus sylvestris*'. *Environmental Pollution*, 21:401-411.
- El-Bassiony, A.M.** 2006. 'Effect of potassium fertilization on growth, yield and quality of onion plant'. *Journal Applied Science Research*, 2(10): 780-785.
- Farooq, M., S.M.A. Barsa and H. Ur-Rahman.** 2006. 'Seed priming enhances emergence, yield, and quality of direct seeded rice'. *Crop Management and Physiology*, 12:42-47.
- Farooq, M., S.M.A. Basra and N. Ahmad.** 2007. 'Improving the performance of transplanted rice by seed priming'. *Plant Growth Regulation*, 51:129-137.
- Frett, J.J., W.G. Pill, D.C. Moreau.** 1991. A comparison of priming agents for tomato and asparagus seed'. *Horticultural Science*, 26:1158-1159.
- Fu, J.R., X.H. Lu, R.Z. Chen, B.Z. Zhang, Z.S. Liu, Z.S. Li and D.Y. Cai.** 1988. 'Osmoconditioning of peanut *Arachis hypogaea* (L.) seeds with PEG to improve vigour and some biochemical activities'. *Seed Science and Technology*, 16:197-212.
- Gao, Y.P., L. Young, P. Bonham-smith and L.V. Gusta.** 1999. 'Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions'. *Plant Molecular Biology*, 40:635-444.
- Ghassemi-Golezani, K., A. Chadordooz-Jeddi, S.Nasrollahzadeh and M. Moghaddam.** 2010. 'Effects of hydro-priming duration on seedling vigour and grain yield of pinto bean (*Phaseolus vulgaris* L.) cultivars'. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38 (1):109-113.
- Ghassemi-Golezani, K., A. Chadordooz-Jeddi and P. Zafarani-Moattar.** 2011. 'Influence of salt-priming on mucilage yield of Isabgol (*Plantago ovata* Forsk) under salinity stress'. *Journal of Medicinal Plants Research*, 5(14):3236-3241.
- Giri, G.S. and W.F. Schilinger.** 2003. 'Seed priming winter wheat for germination, emergence and yield'. *Crop Science*, 43:2135-2141.
- Grieve, C. and M.H. Fujiyama.** 1987. 'The response of two rice cultivars to external Na<sup>+</sup>/Ca<sup>2+</sup> ratio'. *Plant Soil*, 103: 250-345.
- Ikem, A. and N.O. Egibor.** 2005. 'Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) in Georgia and Alabama (United States of America)'. *Journal of Food Composition Analy*, 18:771-787.
- Ikic, I., M. Maric evic, S. Tomasovic, J. Gunjaca, Z.S. Atovic and H.S. Arcevic.** 2012. 'The effect of germination temperature on seed dormancy in Croatian-grown winter wheats'. *Euphytica*, 188:25-34.

- ISTA (International Seed Testing Association).** 2009. International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.
- Kalsa, K.K. and B. Abebie.** 2012. 'Influence of seed priming on seed germination and vigour traits of *Vicia villosa* ssp. *dasycarpa* (Ten.)'. *African Journal of Agricultural Research*, 7(21):3202-3208.
- Kotowski, F.** 1926. 'Temperature relation to germination of vegetable seed'. *Proce. American Society Horticultural Science*, 23:179-184.
- Kummerová, M., S. Zedulka, K. Král'ová and E. Masarovicová.** 2010. 'Effect of zinc and cadmium on physiological and production characteristics in *Matricaria recutita*'. *Biologia Plantarum*, 54:308-314.
- Krantev, A., R. Yordanova and L. Popova.** 2006. 'Salicylic acid decreases Cd toxicity in maize plants'. *General and Applied Plant Physiology*, 45-52.
- La Rocca, N., C. Andreoli, G.M. Giacometti, N. Rascio and I. Moro.** 2009. 'Responses of the Antarctic microalga *Koliella antartica* (Trebouxiophyceae, Chlorophyta) to cadmium contamination'. *Photosynthetica*, 47:471-479.
- Lopez-Millan, A.F., R. Sagardoy, M. Solanas, A. Abadia and J. Abadia,** 2009. 'Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics'. *Environmental and Experimental Botany*, 65, 376-385.
- Marshner, P.,** 2012. Marschner's Mineral Nutrition of Higher Plants, third ed. Academic Press, London, UK.
- Metwally, A., I. Finkemeier, M. Georgi and K.J. Dietz.** 2003. 'Salicylic acid alleviates the cadmium toxicity in barley seedlings'. *Plant Physiology*, 132:272-281.
- Mirshekari, B.,** 2012. 'Seed priming with iron and boron enhances germination and yield of dill (*Anethum graveolens*)'. *Turkish Journal of Agriculture and Forestry*, 36:27-33
- Mishra, A. and M.A. Choudhuri.** 1999. 'Effects of salicylic acid on heavy metal induced membrane degradation mediated by lipoxygenase in rice'. *Biologia Plantarum*, 42:409-415.
- Mishra, S., S. Srivastava, R.D. Tripathi, R. Govindarajan, S.V. Kuriakose and M.N.V. Parasad.** 2006. 'Phytochelatin synthesis and response of antioxidant during cadmium stress in *Bacopa monnieri* L'. *Plant Physiology and Biochemistry*, 44(1):25-37.
- Morsy, F.M., S.H.A. Hassan and M. Koutb.** 2011. 'Biosorption of Cd (II) and Zn (II) by *Nostoc commune*: Isotherm and Kinetics Studies'. *Clean-Soil Air Water*, 39: 680-687.
- Moussa, H. and S. El-Gamal.** 2010. 'Effect of salicylic acid pretreatment on cadmium toxicity in wheat'. *Biologia Plantarum*, 54:315-320.
- Miyadate, H., S. Adachi, A. Hiraizumi, K. Tezuka, N. Nakazawa, T. Kawamoto, K. Katou, I. Kodama, K. Sakurai, H. Takahashi, N. Satoh-Nagasawa, A. Watanabe, T. Fujimura and H. Akagi.** 2011. 'OsHMA3, a P18-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles'. *New Phytologist*, 189:190-199.
- Pandra, S.K. and H.K. Patra.** 2007. 'Effect of salicylic acid potentiates cadmium-induced oxidative damage in *Oryza sativa* L. Leaves'. *Acta physiologiae Plantarum*, 29:567-577.
- Popova, L., L. Maslenkova, R. Yordanova, A. Krantev, G. Szalai and T. Janda.** 2008. 'Salicylic acid protects photosynthesis against cadmium toxicity in pea plants'. *General and Applied Plant Physiology (Special issue)*, 34 (3-4): 133-144.
- Preece, J.E. and P.E. Read.** 1993. Mineral nutrition In: *The biology of Horticulture crop*. 2nd ed., John Wiley and Sons Publisher, 257-259.
- Rama, M.P., J.A. Alonso, C.H. López and E.T. Vaamonde.** 2002. 'Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*'. *Bioresource Technology*, 84(3):265-270.
- Rascio, N. and F. Navari-Izzo.** 2011. 'Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting?'. *Plant Science*, 180(2):169-181.
- Ruan, S., Q. Xue and K. Tylkowska.** 2002. 'Effects of seed priming on germination and health of rice (*Oryza sativa* L.) seeds'. *Seed Science and technology*, 30:451-458.
- Senaratna, T., D. Touchel E. Bumm and K. Dixon.** 2000. 'Acetyl salicylic acid induces multiple stress tolerance in bean and tomato plants'. *Plant Growth Regulation*, 30: 157-161.

- Shafiq, M.** and **M.Z. Iqbal.** 2005. 'The toxicity effects of heavy metals on germination and seedling growth of *Cassia siamea* Lamark'. *Journal of New Seeds*, 7: 95-105.
- Sharikova, F., A. Sakhabutdinova, M. Bezrukova, R. Fatkhutdinova and D. Fatkhudinova.** 2003. 'Changes in the hormonal status of wheat seedling induced by salicylic acid and salinity'. *Plant Science*, 164:317-322.
- Shi, G.R., Q.S. Cai, Q.Q. Liu and L. Wu.** 2009. 'Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes'. *Acta Physiologiae Plantarum*, 31: 969-977.
- Siebert, E.T. and M.D. Richardson.** 2002. 'Effects of osmopriming on Bermuda grass germination and establishment'. *Horticultural Studies, AAES Research Series*, 506:36-38.
- Siswanto, D., P. Suksabye and P. Thiravetyan.** 2013. 'Reduction of cadmium uptake of rice plants using soil amendments in high cadmium contaminated soil: A Pot Experiment'. *The Journal of Tropical Life Science*, 3(2):132-137.
- Sivritepe, N., H.O. Sivritepe and A. Eris.** 2003. 'The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions'. *Scientia Horticulturae*, 97(3-4):229-237.
- Taiz, L. and E. Zeiger.** 2002. *Plant Physiology*. 3<sup>rd</sup> ed, Sinaure Associates, Inc. Publishers, Su and Land, Massachusetts.
- Turk, M. A., A. R. M. Tahawa and K. D. Lee.** 2004. 'Seed germination and seedling growth of three lentil cultivars under moisture stress'. *Journal of Plant Science*, 3: 394-397.
- Wahid, A., A. Noreen, S.M.A. Basra, S. Gelani and M. Farooq.** 2008. 'Priming-induced metabolic changes in sunflower (*Helianthus annuus*) achenes improve germination and seedling growth'. *Botanical Studies*, 49: 343-350.
- Varier, A., A. K. Vari and M. Dadlani.** 2010. 'The sub-cellular basis of seed priming'. *Journal of Current Science*, 99: 450-456.

