Effect of uranium on seed germination and seedlings of sunflower and sorghum

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(Received : September 04, 2016/Accepted : November 12, 2016)

ABSTRACT

The presence of heavy metals is one of the most important environmental stresses. Uranium is the heaviest trace element in nature. To investigate the effects of uranium on the germination and growth of sunflower and sorghum seeds, laboratory testing was conducted in a completely randomized design with four replications. Various concentrations of uranium were 0, 50, 100, 200, 400 and 800 ppm. The results showed that levels of uranium on all traits had a significant effect (1%). The results of analysis of variance for all traits except germination rate and germination percentage showed that sunflower and sorghum were significant at 1%. Seedling length, shoot length and seedling vigour index had the largest decrease with increasing concentrations of uranium. The comparison means showed that the sunflower was more tolerable on various levels of uranium especially at higher levels. A significant correlation was observed between germination percentage and rate of germination. Also a significant correlation was observed between vigour and seedling.

Key words: Seed germination, seedling vigour index, sunflower, uranium

INTRODUCTION

Irrespective of the source, heavy metals have toxic effects on plants and heavy metal known to interfere the environment. Vegetation in the vicinity of polluted area is known to accumulate quite high level of heavy metal (Banerji and Kumar, 1979). Inhibition of seeds germination and retardation of plant growth are commonly observed due to toxicity of heavy metals (Stephan et al., 1991). The development of nuclear science and technology has led to environmental contamination caused by radio nuclides, in particular by uranium (Gavrilescu et al., 2009). Plants also possess the potential to take up DU present in soil and associated water bodies (Neves et al., 2008). Plants growing in contaminated areas were reported to take up to 100 times more U compared to plants of other areas (Alloway, 1990). Uranium in soil does not often create a radiological hazard to humans, but can cause toxicity to plants (Sheppard and Thibault, 1992). The dangers arising from the biochemical toxicity of U as a heavy metal are considered to be about six times higher than those from its radioactivity (Schnug et al., 2005). The information on the U phytotoxicity is yet contradictory; levels as low as 5 mg/kg in soil have been considered as toxic, whereas many studies reported absence of toxicity at U levels 100 to 1000-fold higher (Sheppard and Thibault, 1992).

Little information is available on the accumulation of U in plants grown in U contaminated soils. It is generally observed that plants also vary greatly in their U uptake capacities (Duquene et al., 2006). Moreover, uranium behaviour in soils is controlled by actions and interactions between physico-chemical and biological processes that also determine its bioavailability (Laroche et al., 2005). The solubility of uranium in soil is dependent on several factors such as : pH, redox potential, temperature, soil texture, organic and inorganic compounds, moisture and microbial activity (Rivas, 2005). Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage. In addition, plants
accumulate metal ions that disturb cellular ionic homeostasis. The accumulation of toxic metals in soil and aqueous environments has potential health hazards for humans. Because metals do not degrade in the environment in contrast to organic compounds, remediation must involve either immobilization or removal. Along with metal toxicity, other factors such as arid conditions, a lack of soil structure, low water supply and nutrient deficiency, limit plant growth in contaminated soils. Therefore, promotion of plant growth under stress conditions is critical to the optimum performance of phytoremediation of metal accumulating crops (Belimov et al., 2004). The use of plants to extract metals and radionuclides from contaminated soil and water has been explored as an economical approach (Achakzai et al., 2012). Phytoextraction (uptake) is the use of living green plants in order to remove inorganic contaminants, primarily metals, from polluted soils and concentrate them into roots and easily harvestable shoots (Lasat, 2002; Tang et al., 2003). Phytoremediation can be used to remove not only metals (e. g. Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb and Zn) but also radionuclides (e. g. 90 Sr, 137 Cs, 239 Pu, 234 U, 238 U) and certain organic compounds (Andrade and Mahler, 2002). Plants have shown the capacity to withstand relatively high concentration of contaminants without toxic effects. Some of the heavy metals at low doses are essential micronutrients for plants, but in higher doses, may cause metabolic disorder and growth inhabitation for most of the plant species (Sinha et al., 2005). Researchers have observed that some plant species are endemic to metal-ferrous soil and can tolerate greater than the used amount of heavy metal or other compounds (Peralta et al., 2001). The objective of this work was to study the effect of DU on seed germination and growth in sunflower and sorghum and to select the best plant for phytoremediation.

**MATERIALS AND METHODS**

Experiment was carried out at radio chemistry laboratory of nuclear institute (IRWA) in Iran. The seeds of sunflower (*Helianthus annuus*) and sorghum (*Sorghum bicolor*) were surface sterilized using 5% sodium hypochlorite solution for 5 min and rinsed thoroughly in distilled water. Twenty-five uniform seeds were placed on two layers Whatman No.1 filter paper discs in petri plates (90 × 15 mm) that were moistened with fixed amount of freshly prepared solutions of various concentrations of uranyl nitrate [UO₂(NO₃)₂·7H₂O]. The uranyl nitrate concentrations were 0, 50, 100, 200, 400 and 800 ppm. Petri plates were supplied with 10 ml of each treatment solution and control (distilled water) (Ahmad et al., 2012). A randomized factorial design with four replications was used. Petri dishes were completely covered with a polyethylene sheet to avoid the loss of moisture of evaporation. Petri dishes were placed at 25°C in germinators. Treatment application continued every other day and germination count was started after 48 h of sowing and continued until the 10th day. The seeds were considered to have germinated when their radicle length was at least 2 mm. After 10 days of treatment, plants were harvested and washed immediately. After washing, the lengths of plumule and radicles and seedlings were measured and for the determination of their dry weights, they were wrapped in paper bags and dried in an oven at 70°C for 48 h to a constant dry weight. The final germination percentage, rate of germination and seed vigour index were calculated as follows:

\[
GP = \frac{Ni}{N} \times 100
\]

\[
Ni = \text{Number of germinated seeds}
\]

\[
N = \text{Total number of seeds}
\]

\[
GR = \sum \frac{N}{t}
\]

\[
N = \text{Number of germinated seeds in every day}
\]

\[
t = \text{The number of days after placing the seeds in Petri}
\]

\[
VI = \text{Final GP} \times \text{Seedling lengths} \quad (\text{Elias and Copleland, 2001; Kaboli and Sadeghi, 2002}).
\]

**Statistical Analysis**

The research was conducted using randomized factorial design with four replications. The significance of treatments was analyzed by analysis of variance (ANOVA) using SAS software (SAS Institute Inc., Cary, NC) and Excel software.

**RESULTS AND DISCUSSION**

The effects of different uranium concentrations (0, 50, 100, 200, 400 and 800
ppm) on growth parameters were evaluated. The results are shown in Table 1. Variance analyses of investigated parameters showed the existence of highly significant different levels of uranium which influenced seed germination and all traits (Table 1). Uranium stress had a significant effect on all parameters. Also, the C × U interaction term was significant for more parameters. It was non-significant for germination rate and plumule dried weight (Table 1). The difference between plants for all traits except germination percentage and germination rate was significant at 1% level (Table 1). Mean comparison of different concentrations of uranium simple effect showed the decreasing trend with increasing concentrations of uranium in the characters seen. In particular, seed vigour index, seedling length and plumule length were sensitive to different concentrations of uranium (Table 2). Low concentration of uranium in some traits was similar to control. In other words, treatment 50 ppm uranium on seed germination percentage, germination rate, radicle dried weight and radicle length compared to control had no significant effect. Comparisons of mean crop are shown in Table 3. Results showed that plumule length, seedling length, VI (vigour index seed), radicle dried weight and plumule dried weight in sunflower were greater than sorghum. Compared with sorghum, sunflower was better. But in germination percentage and germination rate in sunflower and sorghum, the differences were not significant. The only trait in sorghum which was higher than sunflower was radicle length (Table 3). Comparisons of the mean interaction C × U are shown in figures.

The results showed that sunflower was superior to sorghum under high concentrations of uranium for most of the traits (Figs. 1, 2, 3 and 4). Finally, sunflower was selected as more resistant to high levels of uranium. In general, simple correlation coefficient showed a linear relationship between traits. There was a significant positive correlation between germination percentage and germination rate. Also, there was a significant positive correlation between seedling and vigour index (Table 4). For 400 and 800 ppm concentrations in sorghum showed abnormal seedlings. Despite the fact that Ni application improved seed germination of sunflower seeds at lower concentrations (Nedhi et al. 1990; Madhava Rao

Table 1. Analysis of variance for characters measured under various levels of uranium on sunflower and sorghum

<table>
<thead>
<tr>
<th>Sources of variations</th>
<th>d. f.</th>
<th>Germination percentage</th>
<th>Germination rate</th>
<th>Radicle dried weight</th>
<th>Plumule dried weight</th>
<th>Plumule length</th>
<th>Radicle length</th>
<th>Seedling length</th>
<th>Ratio root/shoot</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop</td>
<td>1</td>
<td>9.18**</td>
<td>0.064**</td>
<td>0.397**</td>
<td>0.137**</td>
<td>7.20**</td>
<td>0.226**</td>
<td>5.00**</td>
<td>0.646**</td>
<td>4.08**</td>
</tr>
<tr>
<td>Uranium</td>
<td>5</td>
<td>218.22**</td>
<td>32.62**</td>
<td>0.044**</td>
<td>0.281**</td>
<td>4.92**</td>
<td>2.65**</td>
<td>14.62**</td>
<td>0.0204**</td>
<td>16.80**</td>
</tr>
<tr>
<td>Crop × Uranium</td>
<td>5</td>
<td>24.08*</td>
<td>0.428*</td>
<td>0.0231**</td>
<td>0.0084**</td>
<td>1.03**</td>
<td>0.069**</td>
<td>1.44**</td>
<td>0.050**</td>
<td>0.887**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>7.05</td>
<td>0.3724</td>
<td>0.0048</td>
<td>0.005</td>
<td>0.037</td>
<td>0.029</td>
<td>0.060</td>
<td>0.0081</td>
<td>0.062</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>3.07</td>
<td>0.37</td>
<td>14.02</td>
<td>12.65</td>
<td>6.42</td>
<td>9.96</td>
<td>5.19</td>
<td>15.12</td>
<td>5.83</td>
</tr>
</tbody>
</table>

NS: Not Significant. *,**Significant at P=0.05 and P=0.01 levels, respectively.

Table 2. Mean comparison of different concentrations of uranium simple effect for sunflower and sorghum using Duncan’s multiple range test

<table>
<thead>
<tr>
<th>Traits</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination percentage</td>
<td>95.5*</td>
<td>94.5*</td>
<td>90.5*</td>
<td>85.5*</td>
<td>84.62*</td>
<td>83.5*</td>
</tr>
<tr>
<td>Germination rate</td>
<td>6.54*</td>
<td>6.41*</td>
<td>5.15*</td>
<td>4.11*</td>
<td>3.95*</td>
<td>3.85*</td>
</tr>
<tr>
<td>Radicle dried weight</td>
<td>0.287*</td>
<td>0.235*</td>
<td>0.100d</td>
<td>0.177e</td>
<td>0.142d</td>
<td>0.103d</td>
</tr>
<tr>
<td>Plumule dried weight</td>
<td>0.868*</td>
<td>0.761b</td>
<td>0.583f</td>
<td>0.422d</td>
<td>0.453d</td>
<td>0.441d</td>
</tr>
<tr>
<td>Plumule length</td>
<td>2.42*</td>
<td>2.33c</td>
<td>1.90c</td>
<td>1.48e</td>
<td>1.11d</td>
<td>1.15e</td>
</tr>
<tr>
<td>Radicle length</td>
<td>4.08*</td>
<td>3.79*</td>
<td>2.97c</td>
<td>2.71d</td>
<td>2.40e</td>
<td>2.08f</td>
</tr>
<tr>
<td>Seedling length</td>
<td>0.59ab</td>
<td>0.62b</td>
<td>0.65b</td>
<td>0.58ah</td>
<td>0.51b</td>
<td>0.63a</td>
</tr>
<tr>
<td>Ratio root/shoot</td>
<td>6.51*</td>
<td>6.12b</td>
<td>4.9*</td>
<td>4.2d</td>
<td>3.51*</td>
<td>3.23f</td>
</tr>
<tr>
<td>VI</td>
<td>6.21*</td>
<td>5.78b</td>
<td>4.43c</td>
<td>3.54*</td>
<td>3.00b</td>
<td>2.75f</td>
</tr>
</tbody>
</table>

Means followed by similar superscripts do not differ significantly based on Duncan’s multiple range test at P=0.05 level.
Table 3. Mean comparison of crop simple effect using Duncan’s multiple range test

<table>
<thead>
<tr>
<th>Trait</th>
<th>Sunflower</th>
<th>Sorghum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination percent</td>
<td>88.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germination rate</td>
<td>5.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radicle dried weight</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plumule dried weight</td>
<td>0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plumule length</td>
<td>3.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radicle length</td>
<td>1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Seedling length</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ratio root/shoot</td>
<td>5.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>4.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar superscripts do not differ significantly based on Duncan’s multiple range test at P=0.05 level.

and Sresty, 2000). In this study, results showed that U application didn’t improve seed germination of sunflower seeds at lower concentrations. In 50 ppm concentration, some traits such as germination percentage, germination rate, radicle length and radicle dried weight did not have significant difference with control. But the higher levels severely inhibited seed germination and uptake of micro- and macro nutrients such as K and Mg (Nedhi et al., 1990; Madhava Rao and Sresty, 2000).

Results demonstrated that the higher concentration of the uranium pregnant solution could inhibit the seed germination rate of four

![Fig. 1. Comparisons of mean interaction under different levels of uranium and crop vigour index (VI). Means followed by similar letters do not differ significantly based on Duncan’s multiple range test at P=0.05 level.](image)

![Fig. 2. Comparisons of mean interaction under different levels of uranium on crop seedling length. Means followed by similar letters do not differ significantly based on Duncan’s multiple range test at P=0.05 level.](image)
crops with stronger inhibiting effect on soybean and corn. Jagativa and Porohit (2006) in their study showed that effects of various uranium concentrations on sunflower were studied by observation of seed germination, analysis of various growth and biochemical parameters.
Influence was also measured in terms of tolerance index, seedling vigour index and grade of growth inhibition. Shoot-root length, shoot-root fresh and dry weight, chlorophyll contents, tolerance index and seedling vigour index showed gradual decrease with an increase in uranium concentrations. A significant positive correlation was observed between applied uranium concentrations and leaf soluble protein contents, total phenol contents and grade of growth inhibition. The results were consistent with the results of this study. The uranyl concentration (10⁻⁴M to 5·10⁻³M) did not influence drastically the germination rate, the weight and length of the biological product of the seeding experiment (Karin Popa et al., 2014). The low concentration (10 and 20 mg/l) of nickel significantly promoted seed germination and improved early seedling growth, while higher levels (40, 20 and 60 mg/l) caused a significant inhibition in germination and resulted in a considerable delay to achieve 50% germination. Although, plumule and radicle length, and fresh and dry weights of germinating seeds were also improved at lower concentrations, they were significantly reduced in all cultivars at higher levels of nickel.

**CONCLUSION**

Lower concentration (50 ppm) didn’t significantly reduce some traits, for example, germination percentage, germination rate, radicle length and radicle dried weight. It was similar to control treatment. But higher levels of uranium significantly reduced other traits. The sunflower was more tolerable on various levels of uranium especially at higher levels.

**ACKNOWLEDGEMENT**

The authors are thankful to Atomic Energy Organization of Iran, for providing support for conducting this study.

**REFERENCES**


