ABSTRACT: Toxicity of heavy metals such as aluminum and their accumulations in the food chain is one of the main environmental issues in agriculture and natural ecosystems. Aluminum is one of the most important factors limiting plant growth and reduces the efficiency of agricultural production in acid soils. Effects of the aluminum chloride (AlCl₃) were studied on morphological and physiological traits in spinach (Spinacia oleracea L. var. Viroflay). For this purpose, attributes that contain plant height (root and shoot), total chlorophyll (Chl a+b) and concentrations of potassium and calcium were studied in a greenhouse. The aluminum toxicity reduced the plant growth, total chlorophyll and calcium concentrations drastically. In the presence of the aluminum, the potassium concentration increased. Due to a significant reduction in plant growth and Ca absorption which is associated with increased concentrations of aluminum, it can be concluded that Viroflay variety of spinach is not resistant to aluminum toxicity.

Keywords: heavy metals; morphological; physiological; resistant; spinach

INTRODUCTION

Aluminum is the second most abundant element after oxygen and silicon that ability to pollute water, soil and the food chain (Bhalerao and Prabhu., 2013). It is estimated that about 40% of the potentially arable lands worldwide are acidic (Kochian et al., 2004). Acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production (Ryan and Delhaize., 2010). There are various Al forms in soils in monomer, polymer or solid phase. Their concentration depends on the hydrolysis rate of the Al compounds. Al species as Al³⁺ and Al₁₃, are the most toxic to plants (Merino et al., 2010). High concentrations of heavy metals are toxic to plants and cause oxidative stress. In such circumstances, these metal may replacing essential elements and disrupt metabolic functions of plants such as the formation of pigments (Ghosh and Singh., 2005). In addition to acidity, aluminum concentration, temperature and concentrations of cations and anions are other factors that can affect the amount of aluminum toxicity (Wang et al., 2006). Factors such as ammonium fertilizers, organic matter, acid rain can also be acidifying that is reason for solubilization of toxic ions of metals such as aluminum, which eventually causes problems in agriculture and natural ecosystems (Vitorello et al., 2005). Root growth is a most sensitive indicator of aluminum toxicity. The first sign of aluminum toxicity is severe stunting and thickening of the roots (Nosko et al., 1988). Numerous toxicity symptoms are also manifested in shoots. Some of the common responses in shoots to Al toxicity are reduction in shoot length, cellular and ultra-structural modifications in leaves, symptoms resemble to phosphorus deficiency, turning mature leaves dark green, stems purple and killing leaf apexes, due to reduced calcium (Ca) transport young leaves curl, decreased photosynthetic activity, chlorosis and foliar necrosis (Wang et al., 2006). Al appears to preferentially impairs thylakoids and photosynthetic electron transport chain in most plants (Chen et al., 2010).
So, with regard to the issue of heavy metals pollution such as aluminum and also due to the lack of information about the reaction of spinach to aluminum toxicity; this study done to investigate the effects of various concentrations of aluminum on absorption of potassium and calcium, chlorophyll content and plant height in both root and shoot organs of Viroflay spinach.

Material and method
A factorial experiment, in a completely randomized design with three replications, was done in greenhouse of Damghan University. The photoperiod was under 14 hour day and 10 hour night with a temperature of 28 ± 3°C in day and 20 ± 3°C in night. Uniform and homogeneous of spinach (Spinacia oleracea L. var. Viroflay) seeds were planted in trays containing vermicompost and peat moss. The seedlings were watered daily. At four leaf stage, seedlings were grown in plastic trays containing half strength Hoagland’s nutrient solution. Aluminum treatment was applied after a week. Aluminum chloride (AlCl₃) is the main compound of aluminum and added to growth medium in three levels of 0, 100 and 200 mM. To measure chlorophyll, some leaf tissue was homogenized with 80% acetone. After centrifugation, extract absorption read with spectrophotometer (UV.2100 pc) in a wavelength of 646 and 663 nm (Lichtenthaler., 1989). To measure the amount of potassium and calcium of fresh ash was used (Donohue and Aho., 1992). The solution absorption read with ICP device (CBC Integra).

Statistical analysis
Experiments were arranged in a randomized complete block design and data were statistically evaluated by Duncan’s multiple range test. All statistic analyses were carried out using the SPSS 23 statistical software.

Results and discussion
Plant Height (roots and shoots)
Analysis of variance (ANOVA) showed significant difference in height of both shoot and root between different treatment of aluminum chloride (Figure 1 and 2).

Al reduces absorption of water and nutrients such as phosphorus and calcium and change Donnan free space. So, the movement of water and nutrients through a plant - from its roots to its shoots reduce that causing decrease in growth rate and biomass of plant (Gupta et al., 2013). One of the first effects of aluminum toxicity is its negative effect on plant growth. Butler et al. (2001) reported that aluminum treatments, decrease shoot height. The first effect of aluminum toxicity is its negative effect on root growth and meiosis. Inhibition of root division is relating to decline mitotic activity in the meristem of root tip (Horst et al., 1982).

Figure 1 The effect of different concentrations of AlCl₃ on shoot height (cm). The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).
Figure 2 The effect of different concentrations of AlCl3 on root height (cm). The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).

It has been reported that aluminum toxicity inhibits root growth on wild graminae and grasses (Poozesh et al., 2007; Poozesh et al., 2010). Studies on soybean plant showed that aluminum toxicity decrease the height of the root and shoot (Haider et al., 2007). The results of these reports are corresponding with our experimental data.

**Total chlorophyll (Chl a+b)**
The total chlorophyll (Chl a + b) in spinach (*Spinacia oleracea* L. var. Viroflay) significantly decreased under Al stress, while it did not exhibit significant reduction between the different treatments of aluminum in terms of impact on the amount of photosynthetic pigments (Figure 3). Considerable studies have showed that, heavy metals including aluminum reduce photosynthesis reduction in many plant species (Jiang et al., 2008). It has been shown that Al decrease in Chl synthesis by restrains the activity of aminolevulinic acid dehydratase responsible for the formation of monopyrrole porphobilinogen (Pereira et al., 2006). In this experiment with increasing Al treatments, the amount of chlorophyll reduced.

Figure 3. Effects of aluminum toxicity on the content of total chlorophyll (Chl a+b). The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).
This result was consistent with the results of Zhang et al (2007) and Radic et al. (2010), who observed that Al decreased total chlorophyll content in soybean (Glycine max L.) and duckweed (lemna minor L.), respectively. The tea (Camellia sinensis L.) plant was shown that physiological parameters such as pigments and photosynthetic rate have declined because of the toxicity (Mukhopadhyay et al., 2012). Al³⁺ ion disable many enzymes of chloroplasts. Enzyme of Delta-aminolevulinic acid dehydratase is needed by plants to synthesis chlorophyll. Mg²⁺ compete with Al³⁺ for connect to active site of enzyme, which leads to inhibiting of chlorophyll synthesis (Pereira et al., 2006; Zheng et al., 2005). Haider et al. (2007) found that Al decreased Chlorophyll that coincided with decline of Mg and Fe absorption.

**Potassium content**

The potassium content was influenced by Al stress, while it increased significantly under Al stress in both root and shoot (Figure 4 and 5). Potassium is one of the most abundant ions in plant cells that forms about 1 – 10% of dry weight of plants (Epstein and Bloom., 2005). Potassium can maintain electrical potential gradient membrane (Cheeseman and Hanson., 1979), cell development (Mouline et al., 2002), opening and closing of stomata (Dietrich et al., 2001). Activities of a number of enzymes, nitrate and sucrose transition and stabilization of intracellular anion is one of the potassium biological functions (Brito and Kronzucker., 2008). Several studies have demonstrated that increasing the Al supply could increase the potassium absorption in plants (Lin., 2010; Jan., 1991 and Macedo and Jan., 2008). These reports are appears to be in agreement with are observations. Also, Thornton et al. (1986) found that low concentrations of aluminum can increasing potassium content while with the raise of aluminum concentrations, the potassium levels slightly reduce. It has been reported that Al resistant genotypes showed higher uptake, influx, and transport of potassium than did the Al sensitive Genotypes (Mariano and Keltjens., 2005). Uptake of the monovalent cation K has been shown to be less inhibited by Al than the divalent cations Ca and Mg (Rengel and Robinson, 1989). Also, it has been showed that in certain cases the K uptake at low concentrations of Al may be even higher than in solutions without Al (Rengel and Robinson, 1989).

![Figure 4. Effects of aluminum toxicity on the potassium content in shoot. The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).](image)
Figure 5. Effects of aluminum toxicity on the potassium content in root. The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).

**Calcium content**

The calcium content was influenced by Al stress, while it decreased significantly under Al stress in both root and shoot (Figure 6 and 7). Calcium is one of the essential elements in plants (White and Broadley., 2003). Calcium is a regulator of growth and development that is essential for many metabolic pathways (Hepler., 2005). Al³⁺ can block the Ca²⁺ channels and affect cell membrane structure and permeability (Plieth, 2005). Calcium enhances root and shoot growth by effect on cell elongation (White and Broadley., 2003). Ryan *et al.* (1997) demonstrated that a high quantity of Ca²⁺ is present in the apoplast, where it maintains cell and tissue viability. Ma *et al.* (2007) indicated that about 85-99% of total Al present in apoplastic content, and Ca²⁺ is displaced from negative binding sites by Al³⁺ in the apoplasm (Kinraide, 1998). Al³⁺ may inhibit the influx of divalent cations as Ca²⁺ into cells more than monovalent cations, but it stimulates the anion cell influxes (Merino *et al.*, 2010). Disruption of calcium (Ca) uptake by Al has long been considered a possible cause of toxicity, and recent work with wheat (*Triticum aestivum* L.) has demonstrated that Ca uptake at the root apex in an Al-sensitive cultivar was inhibited more than in a tolerant cultivar (Ryan and Kochian., 1993). These results agree with our finding.

Figure 6. Effects of aluminum toxicity on the calcium content in shoot. The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).
Figure 7. Effects of aluminum toxicity on the calcium content in root. The same and different letters show no significant and significant difference, respectively (P<0.05). (Values are mean of three replicates ± SE).

In conclusion, our results demonstrate that calcium may play a role Al tolerance in Viroflay spinach. Essential elements may enhance plant tolerance to Al toxicity. Due to a significant reduction in plant growth and Ca absorption which is associated with increased concentrations of aluminum, it can be concluded that Viroflay variety of spinach is not resistant to aluminum toxicity.

REFERENCES


