Article

Effect of different levels of fluoride on Almond (*Prunus Amygdalus*) seedling

Azadeh Behrooz
Department of Horticulture, College of Abouraihan, University of Tehran, Tehran, Iran
E-mail: azadehbehrooz1981@gmail.com

Received 27 January 2019; Accepted 15 February 2019; Published 1 September 2019

Abstract
The aim of this study was to evaluate the effects of different sodium fluoride concentrations on the growth and certain metabolic parameters of almond seedlings (*Prunus Amygdalus*) under strictly controlled growth conditions in nutrient solutions containing increasing sodium fluoride concentrations ranging from 0 to 9 mM. At the 15 days, productions of material were measured as dry matter was significantly reduced in the root system, which accumulated large amounts of fluoride. As data obtained from this study, the chlorophyll, calcium, and magnesium content of the leaves showed a significant decrease, and the leaf content of starch and sugar was also reduced, especially at the higher fluoride concentrations. Data showed that the mineral concentration changes in the roots were minor except for manganese, which showed a major decrease at 3 mM sodium fluoride. In conclusion we could demonstrate that the nutritional status of the leaves appeared to be affected more than that of roots.

Keywords sodium fluoride; *Prunus Amygdalus*; chlorophyll; seedling.

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction
Fluorine occurs in the atmosphere as gaseous molecules and in reduced form as particulate fluoride (Aoun et al., 2018). In water, inorganic fluorides usually remain in solution under conditions of relatively low pH and hardness (Singh et al., 2018). Plants are exposed to fluoride through the air, soil, and water. Use of this kind of water for irrigation can result in toxicity symptoms on sensitive plants (Neelam et al., 2018). Fluoride is phytotoxic through altering a series of metabolic pathways for most plants (Arnesen, 1998; Bassham, 1979). It can be deposited into soil from several anthropogenic sources, both directly through phosphate fertilizers or indirectly through atmospheric pollution from industrial activities and burning of fossil fuels (Davison and Weinstein, 1998). The symptoms of fluoride toxicity in plants are necrotic regions, especially at the tips and along margins of leaves (Camargo, 2003). Some plants that are more susceptible to fluoride toxicity are monocots, including spider plant, lilies, spikes and dracaena. Furthermore, some of these crops also have long

cropping times and therefore will be irrigated with fluorinated water by growers for months, increasing the risk of developing fluoride toxicity (Fung et al., 1999).

From the soil, fluoride is absorbed by plant roots and then transported via xylematic flow to the transpiratory organs (Stevens et al., 1997), mainly the leaves, where it can accumulate with adverse effects that have been described in the literature (Hewitt, 1966; Horntvedt, 1997; Keller, 1980). The rate at which symptoms appear depends on many environmental factors, such as the type and concentration of pollutants, distance from the emission source, length of exposure, and meteorological conditions (Krupa, 2001). In general, soil fluoride is not available to plants (Klumpp et al., 1998). Roots take up small amounts of soil fluoride by diffusion, which results in a low background concentration in the plant foliage. There are exceptions such as tea plants that are natural accumulators of fluoride (Kim et al., 2003). Gaseous uptake of fluoride by leaves is rapid due to its high solubility. *Prunus Amygdalus* is originating from North Africa and West Asia. It is considered to be one of the most nutritious nuts. Packed with nutrients and beneficial compounds, Almonds help maintain heart and skin health. They are used widely across the globe as a principal ingredient in many healthcare and skincare products. Owing to its high adaptability to semi-arid area climatic conditions and because of the high nutritional value and widening market of its product, the almond tree is receiving increased agricultural attention in Iran. In this report we record changes in some eco-physiological parameters of the fluoride sensitive almond plant, Prunus Amygdalus following treatment of almond seedlings with fluoride under controlled conditions (Miller, 1993; Mezghani et al., 2005). Because of no previous study has been made about this subject this work, therefore, provides a laboratory study on the effects of fluoride on certain metabolic activities of almond *Prunus Amygdalus* seedlings.

2 Materials and Method

Almond seeds (*Prunus Amygdalus)*, were germinated on wet filter paper, in the darkness at 4ºC for 16–22 days (Hewitt, 1966). Following their germination, seedlings of approximately the same size were transferred to a Lang-Ashton nutritive medium that was continuously aerated and renewed in every 7 days. The seedlings were cultivated in a growth chamber under the following conditions: 25-21°C day-night temperature, 16-8 hr photoperiod, and 320 µmol photons per m^2 per second light intensity. After a 14 days acclimatization phase, sodium fluoride was added to the nutritive medium at concentrations of 1, 3, 6, and 9 mM . On the 14th day after the start of fluoride exposure, ten plants from each treatment were harvested. Plant organs were separated into leaves, stems, and roots. Dry matter of the different tissues was determined after drying in an oven at 80ºC for 48 hr and also dried samples were then ground to a fine powder. Powdered plant samples were ashed at 560ºC for 1 hr with 4 g of a sodium potassium carbonate mixture, and the temperature was raised to 960^{oC} for an additional 30 min. The cooled ashed material was then dissolved in 20 mL of 1 M HCl, filtered into a volumetric flask, and the volume was diluted to 60 mL with dematerialized water (Troll et al., 1977). To potentiometric measurement of total fluoride, the diluted solution supernatant was mixed with TISAB-buffer solution (1:10) to dissociate fluoride complexes, stabilize the pH, and maintain a constant ionic strength (Adriano, 1982).

2.1 The chlorophyll concentration evaluation

The chlorophyll concentration in the ground leaves was determined according to the method of (Moran and Porath, 1980).

2.2 Leaf sugar and starch

Reducing sugars in the ground leaves were analyzed according to the procedure of (Ashwell, 1957) and leaf starch was determined according to the method of (Mc Cready et al., 1957). The dried leaves and roots were wet-ashed in a 2:1 v/v nitric and perchloric acid mixture, and Ca, Mg, K, and Fe were determined by atomic absorption spectrophotometry. Each determination was repeated at least twice.

2.3 Statistic analysis

The significant differences between the controls and treatments were determined using the Duncan (1995) test.

3 Results

Data showed that after 14 days of exposure to fluoride in nutrient solution, the dry weight of the leaves and roots of the almond seedlings decreased with increasing fluoride concentration which was significant only for the roots at 6 and 9 mM sodium fluoride (Table 1).

Table 1 Effect of different fluoride concentrations on the dry weight mg of roots and leaves of almond seedlings after 14 days of fluoride exposure.

Sodium fluoride mM	Leaf	Root
0	380	170
1	300	160
3	330	150
6	350	145**
9	345	140**

Values are means SE (n = 8); **p=0.005.

The fluoride concentration increased significantly in both the leaves and roots of the almonds seedlings. The fluoride concentration in roots, however, increased considerably more than in the leaves, ranging from 600 to 1700 µg per g dry matter in the roots and from 25 to 80 µg per g dry matter in the leaves (Table 2).

Table 2 Fluoride accumulation in roots and leaves of almond seedlings after 14 days of exposure to various fluoride concentrations.

Sodium fluoride mM	Leaf	Root
0	25	600***
1	42	800***
3	51	1000***
6	64	1300***
9	80	1700***

***p=0.001.

The chlorophyll content of the leaves was significantly lower at 1 mM fluoride but then showed a small none-significant increase with increasing nutrient fluoride concentration (Table 3).

Table 3 Effect of different fluoride concentrations on chlorophyll content of almond seedling leaves after 14 days of exposure.

Sodium fluoride mM	Chlorophyll content
0	4.1
1	2***
3	2.4***
6	2.5***
9	2.8***

Values are means SE (n = 6). ***p=0.001.

The carbohydrate metabolism showed a decrease in reducing sugars and starch in the leaves with increasing fluoride concentration in the nutrient solution (Table 4).

Table 4 Effect of different fluoride concentrations on leaf reducing sugars contents and leaf starch contents.

Sodium fluoride mM	Reducing sugars	Starch
0	80	1220
1	78	1150
3	65	1210
6	52	1050
9	38	952

Values are means SE (n = 6).

4 Discussion

This investigation demonstrated that fluoride is taken up through the roots of almond seedlings. However, only 6 to 9 percentage of the absorbed fluoride seems to be transported to the leaves. Thus, fluoride retention by the roots might be a mechanism for fluoride tolerance operating in almond root cells. High F accumulation in roots compared to that found in leaves has been reported in previous studies (Weinstein and Alscher, 1977; Giannini et al., 1988). Since formation of reducing sugars such as glucose, fructose, and mannose in leaves is thought to be inhibited by fluoride, the tendency of plants exposed to fluoride to decrease the concentrations of such sugars in their leaves indicates the possible conversion of these sugars to non-reducing sugars, such as sucrose and raffinose or sugar alcohols. Under these conditions, increased levels of non-reducing sugars in tissues might be a mechanism adopted by plants to reduce fluoride toxicity (Singh et al., 2018).

Some researcher showed that the addition of fluoride to the nutrient media also caused various changes in Mg^{2+}, Ca^{2+}, and Fe^{2+} levels in almond seedling leaves. Such changes in mineral content might be expected to induce secondary effects on plant metabolism (Arnesen, 1998). These results suggest that fluoride may not interfere with the translocation of these nutrients to the leaves, although Mn uptake was depressed in the roots at 2.5 mM fluoride (Neelam et al., 2018). Roanz et al. (2004) noted that the fluoride uptake by some plants, which are inherently able to accumulate large quantities of fluoride, was affected both by pH and by Ca levels in the medium (Arnesen, 1998). The reduced F uptake following Ca application appeared not to be due simply to the precipitation of CaF_2 in solution and soil or to the complexing of Ca and fluoride in roots, although these factors cannot be dismissed. It was more likely due to the effect of Ca on the properties of cell wall or membrane permeability in the solution experiments, and to alteration of fluoride speciations and their quantities in soil solutions following Ca application (Takmaz and Davison, 1988). As expected, the marked decrease in leaf Mg concentration of almond seedlings treated with F also led to decreased photosynthesis as evidenced by the relatively low levels of sugars found in F-treated seedlings compared to the controls. Leaf Mg content was also decreased significantly, suggesting that the uptake and upward translocation of Mg^{2+} ions could be disturbed by high levels of fluoride. The toxic action of fluoride toward Mg is also thought to involve the inactivation of Mg^{2+} at its sites of physiological activity (Kim et al., 2003). Decrease in Fe^{2+} content in F-treated almond seedlings might have other physiological implications, such as a decrease in ferridoxin necessary in the light-induced oxido-reduction process in photosynthesis leading to a reduction in chlorophyll content. Inhibition of photosynthesis is also expected to occur with lower Mg^{2+} and Fe^{2+} concentrations, and this could explain the relatively low levels of reducing sugars and starch in the leaves (Arnesen, 1998).

5 Conclusion

We could demonstrate that the chlorophyll, calcium, and magnesium content of the leaves showed a significant decrease, and the leaf content of starch and sugar was also reduced, especially at the higher fluoride concentrations and the mineral concentration changes in the roots were minor except for manganese were decrease at 3 mM sodium fluoride. Also the future studies are needed for more explanation.

References

Adriano DC, Doner HE. 1982. Bromine, chlorine and fluorine. In: Methods of Soil Analysis Part II – Chemical and Microbiological Properties. Agronomy Series 9 (Page AL, Miller RH, Keeney DR, eds) (2nd ed). American Society of Agronomy, Soil Science Society of America. 449-483, Madison, Wisconsin, USA

Aoun A, Darwiche F, Al Hayek S, Doumit J. 2018. The fluoride debate: The pros and cons of fluoridation. Preventive nutrition and Food Science, 23(3): 171-180

Arnesen AKM.1998. Effects of fluoride pollution on pH and solubility of Al, Fe, Ca, Mg, K and organic matter in soil from Ardal (Western Norway). Water Air and Soil Pollution, 103: 375-388

Ashwell G. 1957. Colorimetric analysis of sugars. In: Methods in Enzymology Vol. III (Colowick SP, Kaplan NO, eds). 85-86, Academic Press, New York, USA

Bassham JA. 1979. The reductive pentose phosphate cycle and its regulation. In: Encyclopaedia of Plant Physiology. New Series Vol. 6 (Gibbs M, Latzko E, eds). 9-30, Springer-Verlag, Berlin, Germany

Camargo JA. 2003. Fluoride toxicity to aquatic organisms: a review. Chemosphere, 50: 251-264

Davison A, Weinstein LW. 1998. The effects of fluorides on plants. Earth Island Journal, 13: 257-264

Fung KF, Zhang ZQ, Wong JWC, Wong MH. 1999. Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. Environmental Pollution, 104: 197-205

Giannini JL, Miller GW, Pushnik JL.1998. Effects of NaF on biochemical processes of isolated soybean chloroplasts. Fluoride, 18 (2): 72-79

Hewitt EJ. 1966. Sand and water culture methods used in the study of plant nutrition. Technical Communication GB: Commonwealth Bureau of Horticulture Plantation Crops. Commonwealth Agric. Bureau, UK

Horntvedt Hogskolevein R. 1997. Accumulation of airborne fluorides in forest trees and vegetation. Fluoride, 30(3): 188

Keller T. 1980. The simultaneous effect of soil-borne NaF and air pollutant SO_2 uptake and pollutant accumulation. Oecologia, 44: 283-285

Kim CG, Power SA, Bell JNB. 2003. Effects of cadmium and soil type on mineral and carbon partitioning in seedlings of Pinus sylvestris. Water Air and Soil Pollution, 145: 253-266

Klumpp A, Klumpp G, Domingos M, Silva MD. 1998. Fluoride impact on native tree species of the Atlantic Forest near Cubatão, Brazil. Water Air and Soil Pollution, 78: 57-71

Krupa, S. 2001. Fluorine. In: Encyclopedia of Plant Pathology (Maloy OC, Murray TD, eds). John Wiley, New York, USA

Mezghani I, Elloumi N, Ben Abdallah F, Chaieb M, Boukhris M. 2005. Fluoride accumulation by vegetation in the vicinity of a phosphate fertiliser plant in Tunisia. Fluoride, 38(1): 18-24

Miller GW. 1993. The effect of fluoride on higher plants: with special emphasis on early physiological and biochemical disorders. Fluoride, 26: 3-22

Moran R, Porath D. 1980. Chlorophyll determination in intact tissues using N, N-dimethyl formamide. Plant Physiology, 65: 478-479

Neelam Y, Khushboo RSS, Yadav DK, Yadav VK, et al. 2018. Soil and water pollution with fluoride, geochemistry, food safety issues and reclamation. International Journal of Current Microbiology and Applied Sciences, 7(5): 1147-1162

Ruan J, Ma L, Shi Y, Han W. 2004. The impact of pH and calcium on the uptake of fluoride by tea plants. Annals of Botany, 93(1): 97-105

Singh G, Kumari B, Sinam G, Kriti, Kumar N, Mallick S. 2018. Fluoride distribution and contamination in the water, soil and plants continuum and its remedial technologies, an Indian perspective- a review. Environmental Pollution, 239: 95-108

Stevens DP, McLaughlin MJ, Alston AM. 1997. Phytotoxicity of aluminum- fluoride complexes culture by Avena sativa and Lycopersicon esclentum. Plant and Soil, 192: 81-93

Stevens DP, McLaughlin MJ, Alston AM. 1998. Phytotoxicity of the fluoride ion and its uptake from solution culture by avena sativa and Lycopersicon esclentum. Plant and Soil, 200: 119-29

Takmaz Nisancioglu S, Davison AW. 1988. Effects of aluminum on fluoride uptake by plants. New Phytologist, 109: 149-155

Troll G, Farzaneh A, Camman K.1977. Rapid determination of fluoride in mineral and rock samples using an Ion-selective electrode. Chemical Geology, 20: 295

Weinstein LH, Alscher-Herman R. 1977. Physiological responses of plants to fluorine. In: Effects of Gaseous Pollutants in Agriculture and Horticulture (Unsworth MH, Ormrod DP, eds). 139-167, Butterworth, London, UK