BIOMONITORING OF FLUORIDE POLLUTION WITH *GLADIOLUS* IN THE VICINITY OF A BRICK KILN FIELD IN LAHORE, PAKISTAN

Kifayat Ullah,[a] S Razi Abbas Shamsi,[a] S Saeed Ahmad,[b,]* M Nauman Ahmad,[c] Sardar Khan,[d] Rabail Urooj,[b] M Shahid Iqbal,[e] Afia Zia,[c] N Ahmad Khan[f]

Lahore, Pakistan

ABSTRACT: Although there are thousands of small-scale, poorly regulated brick kilns in rural areas of South Asia their impact on local agricultural crops is largely unknown. The impact of fluoride on crops in a brick kiln area in the Northern Punjab Region of Pakistan was investigated. Fluoride accumulation, leaf necrosis, and reduced corm diameter and weight were found in the brick kiln area in two cultivars of *Gladiolus* that have been widely used as biomonitors in Europe. The rate of increase in leaf injury of the sensitive *Gladiolus* cultivar was greatest at all sites when the temperature was lower and there was a high relative humidity of 60–75%. The necrotic leaf tip lengths of the indicator plants correlated very well with their fluoride concentrations. The fluoride accumulation was directly proportional to the Fluoride Injury Index, although a higher injury index was observed with the fluoride-sensitive cultivar Lavendell Puff compared to the fluoride-tolerant cultivar Flower Song. These results suggest that injury to sensitive crops from fluoride may occur in other brick kiln areas in South Asia, and that *Gladiolus* plants could be used as a cost-effective biomonitor for further investigation in this region.

Keywords: Biomonitoring; Brick kilns; Fluoride; *Gladiolus*; Pakistan; South Asia.

INTRODUCTION

Hydrogen fluoride (HF) is a toxic gas emitted from many industries utilizing or manufacturing fluoride compounds.[1] Brick kilns, volcanoes, crevices, vents, hydrothermal systems, and burning timber are the major sources of atmospheric fluoride pollution.[2] HF causes damage to the ecosystem and is one of the most phytotoxic of the air pollutants causing injuries and foliar lesions in plants by altering their metabolic pathways with resulting reductions in growth rate.[2] Sensitive species can be affected at concentrations of approximately 10–1,000 times lower than those of other major pollutants.[3,4,5] The negative effects of fluoride pollution on plants near poorly regulated emission sources have been documented for many years.

High foliar fluoride concentrations have been linked with injury to sensitive plants. Mason et al.[6] reported that visible leaf injury decreased with distance from brick works and that this followed the pattern of leaf fluoride levels in each season. HF fumigation of plants under controlled conditions led to the idea that the increase in fluoride content of plant tissue was related to the concentration in air and the duration of exposure.[6] There is a negligible contribution to leaf fluoride content by uptake through the roots in highly polluted areas, as the direct absorption of airborne fluorides by plant foliage masks any soil uptake.[7]

[a]Botany Department, University of the Punjab, Lahore, Pakistan; [b]Environmental Science Department, Fatima Jinnah Women University, Rawalpindi, Pakistan; [c]Agricultural Chemistry Department, University of Agriculture, Peshawar, Pakistan; [d]Environmental Science Department, University of Peshawar; [e]Environmental Science Department, Wageningen University, Peshawar, Pakistan; [f]Department of Animal Nutrition, University of Agriculture, Peshawar, Pakistan. *For correspondence: S Saeed Ahmad, Environmental Science Department, Fatima Jinnah Women University, Rawalpindi, Pakistan; E-mail: drsaeed@fjwu.edu.pk, Ph: 0092 9270050

Many monocotyledonous species, such as tulips, lilies, and gladioli, are known to be extremely sensitive to fluorides and serve as response indicators of fluoride pollution.[8,9,10] *Gladiolus* has been used as a bioindicator of fluoride pollution in many studies in Europe and North America. Some cultivars of *Gladiolus* have proved to be very sensitive, showing typical necrosis in the tip and margins of leaves (tip burn) at low foliar fluoride concentrations.[11] The overall yield of corms of fluoride-sensitive *Gladiolus* varieties were reduced by 20–40% at a distance of 1.5–2.5 km from a fluoride source.[8]

However, much less research has been conducted on the effects and biological monitoring of fluoride outside Europe and North America. Klumpp et al.[12] reported 9% visible leaf injury to *Gladiolus* plants, with a foliar concentration of about 50 µg/g dry matter (DM), at a site near an industrial source of fluoride pollution in Cubatao, Brazil. Fluoride biomonitoring with *Gladiolus* has not been previously carried out in the South Asia.

In many parts of Asia, brick production is a very large industry, and the demand for bricks is increasing due to rapid socio-economic growth.[13] The total output of bricks in China is approximately 800 billion annually, while the brick industry of India has an estimated production rate of around 140 billion bricks annually.

The main aim of this study was to evaluate the feasibility of using *Gladiolus* plants as fluoride biomonitors in this region. The work was carried out around the city of Lahore, in Pakistan, but is relevant to the wider impacts of fluoride emissions from brick works in South Asia.

METHODOLOGY

Site selection: The field work was carried out around a central cluster of brick kilns, in a rural agricultural area, north of Lahore. The main source of fluoride in this area was a group of 7 brick kilns at Chung Khurd (31º 28’ 50” N, 74º 24’ 50” E). The soil of the investigated area is used as raw material for making bricks.

Fluoride biomonitoring with Gladiolus: Gladiolus corms of two cultivars (Lavendell Puff and Flower Song) were provided by the Research Institute for Agrobiology and Soil Fertility, Wageningen, The Netherlands, for the purpose of fluoride biomonitoring. The cultivar Lavendell Puff was identified as being sensitive to fluoride pollution while the cultivar Flower Song was identified as being resistant.

Gladiolus corms were grown in 25 cm diameter earthen pots. Seedlings of *Gladiolus* cultivars were raised in growth chambers at the Environmental Science Department, University of the Punjab University, Lahore, and were then transferred to the field on day 28 of germination to biomonitor fluoride concentration around the brick kilns. The fluoride biomonitors were exposed in six sites around the central brick kiln area and the control site at the University of the Punjab, Lahore. At each site, six pots of each cultivar, with one plant per pot, were exposed to air in 2.0×1.0×0.6 m^3 expanded metal cages. Individual *Gladiolus* plants were observed weekly from 15 November 2010 to 24 January 2011, when the temperature was lower and the humidity was higher.

The percentage of necrotic leaf area was taken as the index of fluoride injury.[9] The degree of necrosis of the leaf tips and margins was assessed quantitatively by measuring the mean length (cm) of the necrotic tips, as an estimate of the necrotic leaf area. The mean total length (cm) of the exposed leaf blades was also measured. From the values for the mean length of necrotic tips (cm) and the mean total length of the leaf blades (cm), the plant injury index was calculated:

$$\text{Plant injury index (\% of whole plant)} = \frac{\text{Mean injured leaf length of the plants (cm)}}{\text{Mean total leaf length of the plants (cm)}} \times 100$$

On the date of the final observation, standard 7.5 cm leaf tip lengths were cut from each of the *Gladiolus* plants and brought back to the laboratory of the Environmental Science Department, University of the Punjab, Lahore, for fluoride determination, using the standard acid digest method of the AOAC.[14] At the same time, the *Gladiolus* plants were also harvested and brought back to the laboratory. After the newly formed corms were separated from each plant and cleaned to remove soil particles, their weights and diameters were measured.

RESULTS AND DISCUSSION

Leaf tip injury assessment: The relationship between the leaf fluoride concentration and the amount of damage occurring in both cultivars of *Gladiolus* is shown in Figures 1a and 1b. Differential responses were seen in the percentage injury both between cultivars and between sites. Compared to the fluoride-tolerant *Gladiolus* cultivar Flower Song, which showed a maximum leaf injury of approximately 6% (Figure 1a), the fluoride-sensitive *Gladiolus* cultivar Lavendell Puff, placed at the sites to the south (South-I and South-II) of the brick kiln area, showed the greatest leaf injury per plant (20–22%, Figure 1b). The lowest degree of injury for both cultivars was found to the east of the brick kiln area and at the control site.

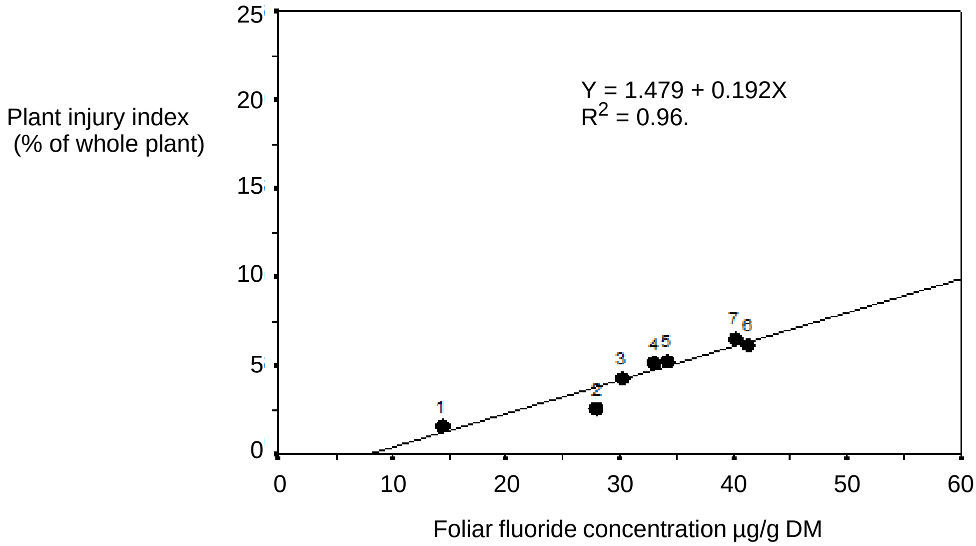

Figure 1a. Effect of fluoride accumulation in the leaf tips of the fluoride-tolerant *Gladiolus* cultivar Flower Song based on the plant injury index. Sites: 1 Control, 2 East, 3 West, 4 North-I, 5 North-II, 6 South-I, 7 South-II. Y = 1.479 + 0.192X; R^2 = 0.96.

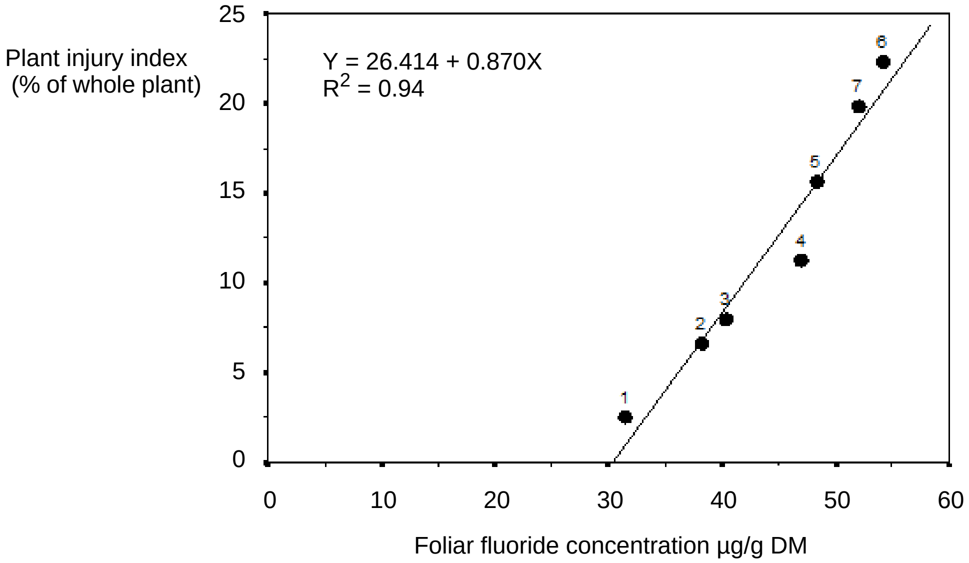

Figure 1b. Effect of fluoride accumulation in the leaf tips of the fluoride-sensitive *Gladiolus* cultivar Lavender Puff based on the plant injury index. Sites: 1 Control, 2 East, 3 West, 4 North-I, 5 North-II, 6 South-I, 7 South-II. Y = 26.414 + 0.870X; R^2 = 0.94.

There was also a marked difference in the fluoride content of the leaf tip samples taken from *Gladiolus* plants grown at the different sites (Figures 1a and 1b). The highest concentration of fluoride, 52–54 µg/g DM was found at the sites in the south of the main brick kiln cluster in Lavendell Puff, whilst the lowest (38 µg/g DM) was found at the site to the east of main cluster. The fluoride contents of the cv. Flower Song were consistently lower than those of the cv. Lavendell Puff, although the trends were the same, with the lowest values occurring at sites to the east and west, and the highest values occurring at sites to the south of the brick kilns. Lavendell Puff also had significantly higher concentrations at the control site.

Figures 1a and 1b show that, in addition to the greater accumulation of fluoride in the sensitive cultivar, the increase in leaf injury per unit increase in foliar fluoride concentrations was much greater in the cv. Lavendell Puff than in the cv. Flower Song; the slopes of the fitted lines differed by a factor of 4–5. These differences were attributed to the differential sensitivities of the two cultivars to fluoride.

Fluoride damage to corms: Figures 2a and 2b show that the diameter of the newly formed corms at harvest decreased with increasing fluoride accumulation in the leaves. The fitted regression lines between foliar fluoride concentration and corm diameter were similar for both the sensitive and tolerant *Gladiolus* cultivars, despite the fact that the cv. Lavendell Puff had higher degrees of leaf injury and higher fluoride concentrations. However, the cv. Lavendell Puff had higher fluoride concentrations overall, and hence, lower corm diameters, than cv. Flower Song.

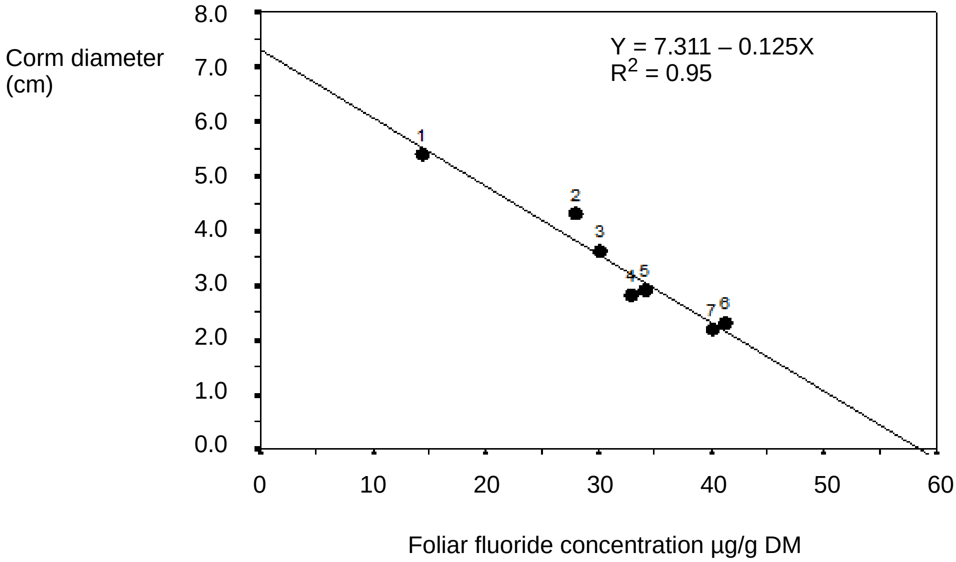

Figure 2a. Effect of fluoride accumulation in the leaf tips of the fluoride-tolerant *Gladiolus* cultivar Flower Song based on the diameter of their respective corms. Sites: 1 Control, 2 East, 3 West, 4 North-I, 5 North-II, 6 South-I, 7 South-II. Y = 7.311 – 0.125X; R^2 = 0.95.

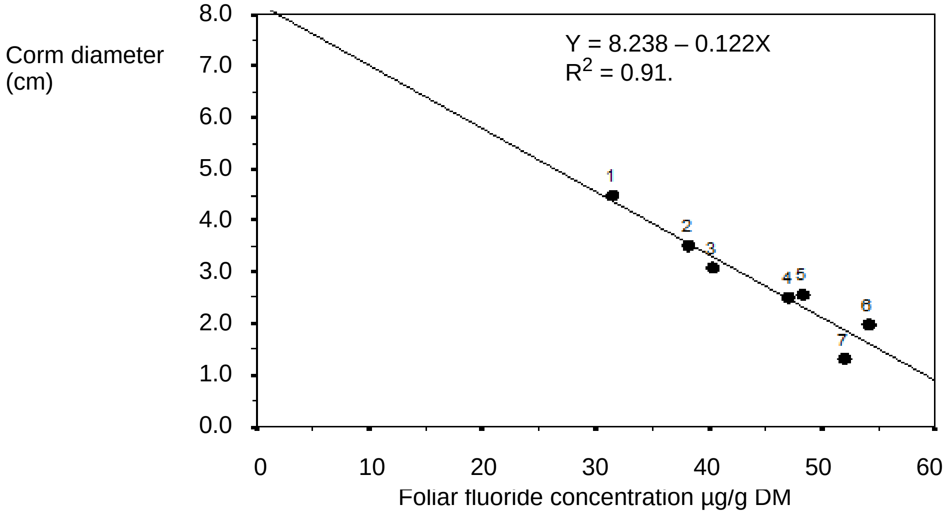

Figure 2b. Effect of fluoride accumulation in the leaf tips of the fluoride-sensitive *Gladiolus* cultivar Lavender Puff based on the diameter of their respective corms. Sites: 1 Control, 2 East, 3 West, 4 North-I, 5 North-II, 6 South-I, 7 South-II. Y = 8.238 – 0.122X; R^2 = 0.91.

Figures 3a and 3b show the relationship between the leaf fluoride levels and the weight of the newly formed corms of *Gladiolus* plants at the time of harvest. The lowest reduction in the weight of newly formed corms was observed at the sites with the lowest leaf fluoride levels, situated in the east and west of the central cluster of kilns. As for corm diameter, the cv. Lavendell Puff had higher fluoride concentrations overall and hence lower corm weights, than the cv. Flower Song. However, the slope of the fitted relationships was greater for the cv. Flower Song, suggesting a greater effect of fluoride accumulation on corm weight in this cultivar.

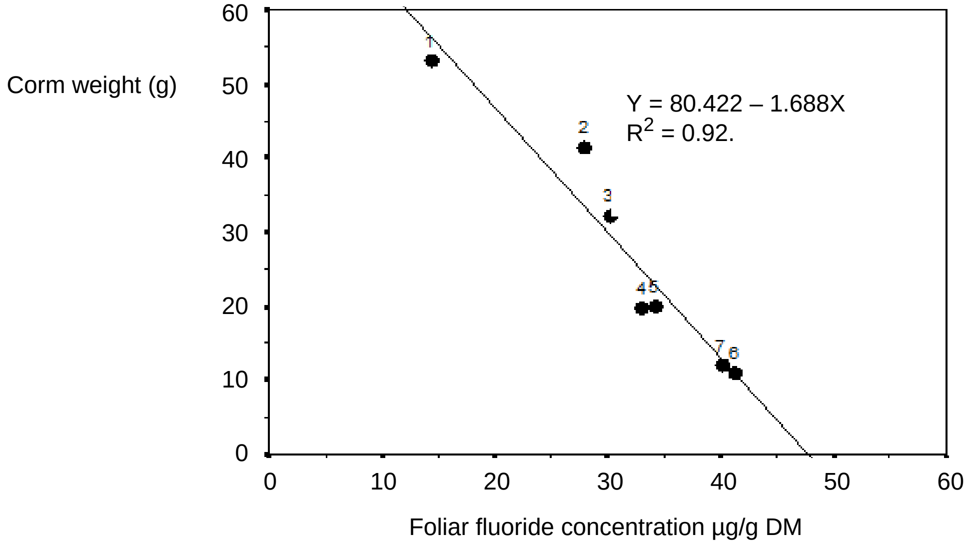

Figure 3a. Effect of fluoride accumulation in the leaf tips of the fluoride-tolerant *Gladiolus* cultivar Flower Song based on the weight of their respective corms. Sites: 1 Control, 2 East, 3 West, 4 North-I, 5 North-II, 6 South-I, 7 South-II. Y = 80.422 – 1.688X; R^2 = 0.92.

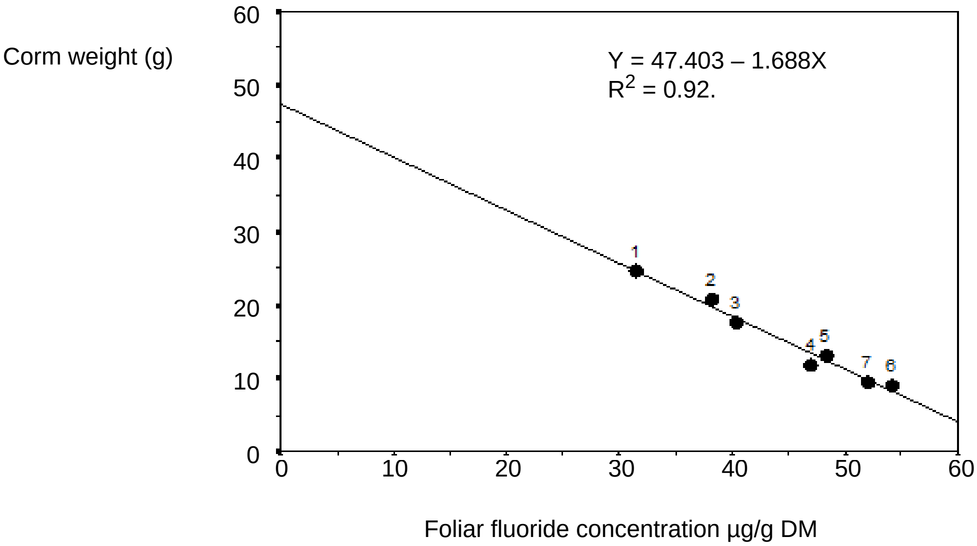

Figure 3b. Effect of fluoride accumulation in the leaf tips of the fluoride-sensitive *Gladiolus* cultivar Lavender Puff based on the weight of their respective corms. Sites: 1 Control, 2 East, 3 West, 4 North-I, 5 North-II, 6 South-I, 7 South-II. Y = 8.238 – 0.122X; R^2 = 0.91.

The rate of increase in leaf injury of the sensitive *Gladiolus* cultivar was greatest at all sites when the temperature was lower and there was a high relative humidity of 60–75%. Our findings are in agreement with MacLean et al. [15] who found that *Gladiolus* fumigated with hydrogen fluoride was more severely injured at 85% relative humidity than at 65% or 50% relative humidity. A very close association was found between the fluoride contents in the leaf tips and the size and weight of *Gladiolus* corms at the end of growing season. These results are in agreement with the finding of an overall corm yield reduction of 20–40% at 1.5–2.5 km from the source in very wet conditions, although the corresponding value was 6–14% in a normal cropping season.[8] The crop yield reduction was higher in the more susceptible cultivars than in the less susceptible cultivars.[8] Posthumus reported a relationship between leaf injury by HF and corm yield reduction.[8] Necrotic leaves adversely affected flower production and corm yield.[16] Necrosis covering 10–70% of the leaf surface reduced flowering and corm yield in *Gladiolus* plants.[16]

Figures 1a and 1b indicate that the plant injury index was increased with higher levels of fluoride accumulation and also increased for the fluoride-sensitive cultivar Lavendell Puff compared to the fluoride-tolerant cultivar Flower Song. The necrotic leaf tip lengths of the indicator plants correlated very well with the fluoride concentrations.[8] Posthumus reported that the leaf tip fluoride content of the fluoride-tolerant *Gladiolus* cultivar Flower Song in The Netherlands was 143 μg/g DM.[8] These results are in line with the results in the current study with the fluoride-tolerant *Gladiolus* cultivar Flower Song where a leaf tip necrosis level of 11% corresponded to a leaf tip fluoride concentration of 60 μg/g DM (Figure 1a). In a fumigation experiment, Coulter et al. reported 27% leaf necrosis on *Gladiolus* cultivar T210 when exposed to 22 μg F/m^3 for 48 hr.[9] These findings are similar to our results in the fluoride-sensitive *Gladiolus* cultivar Lavendell Puff where a of leaf necrosis level of 23% occurred with a leaf tip fluoride concentration of 53 μg/g DM (Figure 1b). Klumpp et al. reported on fluoride-exposed *Gladiolus* plants at Mogi Valley, Brazil, where 7–10%, 9%, and 18–20% leaf injury occurred with leaf tip fluoride levels of 18–20, 50, and 100 μg/g DM, respectively.[7,12] These values are comparable to our findings with the fluoride-sensitive *Gladiolus* cultivar Lavendell Puff of 20% and 10% leaf injury with leaf tip fluoride concentrations of 50 and 45 μg/g DM, respectively (Figure 1b).

CONCLUSIONS

Severe leaf tip necrosis was observed in the fluoride-sensitive Gladiolus cultivar Lavendell Puff exposed to HF in the brick kiln area. In this fluoride-sensitive *Gladiolus* cultivar, a leaf tip fluoride concentration of approximately 40 μg/g DM caused a leaf injury index of 10%, while in the fluoride-resistant cultivar a leaf tip fluoride concentration of 50 μg/ g DM was required to cause the same leaf injury index. Significant reductions in the corm weight and size were also found in the both cultivars of *Gladiolus* in the brick kiln area. It was concluded that the use of *Gladiolus* plants was an effective biomonitoring technique for assessing the impact of atmospheric fluoride concentrations near a small scale, poorly managed brick kiln in South Asia. Ahmad et al. noted that the soil, air, and water in the vicinity of brick kilns may also be affected by other pollutants, such as ozone (O_3), and similar biomonitoring studies should be carried out on them as, currently, no data are available for setting threshold levels for air pollutants like HF and O_3 in Pakistan.[17,18,19]

REFERENCES

1 Weinstein LH, Davison AW. Fluorides in the Environment. Wallingford, Oxon, UK: CABI Publishing CAB International; 2004.
2 Weinstein LH. Fluoride and plant life. J Occup Med 1977;19(1):49-78.
3 Franzaring J, Klumpp A, Fangmeier A. Active biomonitoring of airborne fluoride near an HF producing factory using standardized grass cultures. Atmos Environ 2007;41:4828-40.
4 Wahid A, Ahmad SS, Ahmad MN, Khaliq B, Nawaz M, Shah SQ, Shah RU. Assessing the effects of hydrogen fluoride on mango (*Mangifera indical* L.) in the vicinity of a brick kiln field of Southern Pakistan. Fluoride 2014;47(4):307-14.
5 Ahmad MN, Ahmad SS, Zia A, Iqbal MS, Shah H, Mian AA, Shah RU. Hydrogen fluoride effects on local mung bean and maize cereal crops from peri-urban brick kilns in South Asia. Fluoride 2014;47(4):315-9.
6 Mason MG, Cameron I, Petterson DS, Home RW. Effect of fluoride toxicity on production and quality of wine grapes. Aust Inst Agric Sci 1987;53(2):96-9.
7 Klumpp A, Modesto IF, Domingos M, Klumpp G. Susceptibility of various *Gladiolus* cultivars to fluoride pollution and their suitability for bioindication. Pesq Agro Brasi 1997;32:239-47.
8 Posthumus AC. Higher plants as indicators and accumulators of gaseous air pollution. Environ Monit Assess 1983;3-4:263-72.
9 Coulter, CT Pack MR Sulzback CW. An evaluation of the dose-response relationship of fluoride injury to *Gladiolus*. Atmos Environ 1985;19:1001-7.
10 Rey-Asensio A, Carballeira A. *Lolium perenne* as a biomonitor of atmospheric levels of fluoride. Environ Int 2007;33:583-8.
11 Van Raay A. Biological and chemical diagnosis of damage to crops caused by air pollution and tracing the source of pollution. Annual Report, Institute of Phytopathological Research, Wageningen, The Netherlands. Wageningen, The Netherlands: Institute of Phytopathological Research, Wageningen; 1975.
12 Klumpp A, Domingos M, Klumpp G. Assessment of the vegetation risk by fluoride emissions from fertilizer industries at Cubatao, Brazil. Sci Total Environ 1996;192:219-28.
13 Regional Energy Resources Information Center (RERIC). Small and medium scale industries in Asia: energy and environment; brick and ceramic Sectors. Pathumthani, Thailand: Regional Energy Resource Information Center, Asian Institute of Technology, Pathumthani, Thailand; 2003.
14 Horwitz W, editor. Official methods of analysis. 13th ed. Washington DC; Association of Official Analytical Chemists (AOAC); 1980.
15 MacLean DC, Schneider RE, McCune DC. Effects of chronic exposure to gaseous fluoride on the field-grown bean and tomato plants. J Am Soc Hortic Sci 1973;102:297-9.
16 Brewer RF, Guillement FB, Sutherland FH. The effects of atmospheric fluoride on gladiolus growth, flowering and corm production. Proc Am Soc Hortic Sci 1966;88:631-4.
17 Ahmad MN, Büker P, Khalid S, Van Den Berg L, Shah HU, Wahid A, et al. Effects of ozone on crops in Pakistan. Environ Pollut 2103;174;244-9.
18 Ahmad MN, van den Berg LJL, Shah HU, Masood T, Büker P, Emberson L, Ashmore M. Hydrogen fluoride damage to vegetation from peri-urban brick kilns in Asia: a growing but unrecognised problem? Environ Pollut 2012;162:319-24.
19 Ahmad SS, Murtaza R, Shabir R, Ahmad MN, Shah TA. Environmental diversification and spatial variations in riparianvegetation: a case study of Korang River, Islamabad. Pakistan Journal of Botany 2014;46(4):1203-10.