Tropical Plant Research
An International Journal
© Society for Tropical Plant Research

ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
2(2): 134–142, 2015

Research article

Effect of fluoride contaminated irrigation water on Eco-physiology, biomass and yield in *Gossypium hirsutum* L.

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Abstract: The study was conducted to assess the response of a cash crop *Gossypium hirsutum* L. to the irrigation of fluoride (F) contaminated water. Different concentrations of F *i.e.* 100, 200, 500 and 1000 ppm were used to study the effects of these concentrations of this element on CO_2 assimilation, stomatal conductance, chlorophyll fluorescence, biomass and yield of *G. hirsutum*. Photosynthesis (P_N), stomatal conductance (g_s), chlorophyll fluorescence (Fv/Fm), plant growth and biomass accumulation in terms of fresh and dry weight were decreased significantly with the application of F. The results were concentration dependent. However, a significant increase in harvest index was noticed even at 200 ppm concentration of F. There was no any significant phytotoxic effect noticed on morphology of plant in terms of necrosis, tip burning and curling of leaf. Moreover, the parameters studied were decreased but the crop yield was increased slightly cultivated with the application of F contaminated water, the studied cultivar of this crop may be recommended for the cultivation in the fluoride contaminated zones.

Keywords: Biomass Production - Photosynthetic CO_2 Assimilation - Stomatal Conductance - Chlorophyll Fluorescence - Harvest Index.

[**Cite as:** Kumar S & Singh M (2015) Effect of fluoride contaminated irrigation water on Eco-physiology, biomass and yield in *Gossypium hirsutum* L. *Tropical Plant Research* 2(2): 134–142]

INTRODUCTION

Fluoride (F) occurs naturally in the earth's crust in a very minute amount, but frequently acts as an environmental pollutant (Jacobson *et al.* 1966). It is a common phytotoxicant, frequently present in air, water and soil. It has been commonly released to the environment by a number of anthropogenic activities (EPA 1978, Kamaluddin & Zwiazek 2003). Fluoride has been reported to cause dental and skeletal fluorosis in animals (Raju *et al.* 2009). In case of plant certain physiological processes are known to be markedly affected by F *e.g.* decreased plant growth (Elloumi *et al.* 2005, Jacobson *et al.* 1966), chlorosis (Mcnulty & Newman 1961), leaf tip burn and necrosis Hadujue (1966). The importance of seed germination is widely recognized and its study has been used as a model for investigating F toxicity by various authors (Elloumi *et al.* 2005, Gulzar & Khan 2001, Gupta *et al.* 2009, Rubio-Casal *et al.* 2003, Wang *et al.* 1991, Wilde & Yu 1998).

Fluoride contamination is normally occurred in ground water due to the weathering of parental rocks such as fluorapatite ($Ca_5(Po_4)_3F$), Fluorite (CaF_2), Cryolite (Na_3AlF_6), and fluorophlogopite etc. (Kabata-Pendias & Pendias 1984, Elrashidi & Lindsay 1986, Fuge & Andrews 1988). Ground water is the main source of irrigation for the crop production. Application of fluoride contaminated ground water for irrigation is widespread in many fluoride contaminated areas which can affect growth and productivity of the crops. A number of studies have been reported to evaluate the effects of fluoride on growth, productivity, yield and tolerance of plants (Elloumi *et al.* 2005, Datta *et al.* 2012, Gadi *et al.* 2012, Singh & Verma 2013). Many workers have reported the effect of F on various cereal crops and vegetables using concentration up to 200 ppm. Owing to high adaptability to arid and semi-arid area climatic conditions and because of its high commercial value and widening market of its product, *G. hirsutum* is receiving increase agricultural attention in North India. There is no any study has been conducted on this plant as per our review literature. In the present study, the efforts have been made to evaluate the efficacy of *G. hirsutum* towards the application of fluoride contaminated water used as irrigation. Moreover,

Received: 10 June 2015 Published online: 31 August 2015

the effects of fluoride on photosynthesis, growth, biomass and yield of cotton (*G. hirsutum*) crop have also been studied.

MATERIALS AND METHODS

Plant material

Cultivars PUSA-S-6 of *Gossypium hirsutum* was collected from Krishi Vigyan Kendra, Bulandshahr of Sardar Vallabh Bhai Patel University of Agriculture Technology, Meerut (U.P.), India. The cotton seeds are grown in soil bed and the next 10 days the germination pattern was observed. On 11^{th} days of germination, the saplings were transferred to earthen pots having size 12" filled with 8 kg of soil having 3:1 ratio of soil and compost respectively. The saplings were provided with adequate amount of water and natural condition for next 30 days to get established. Now, the treatment was continued for next 80–90 days and consequently, the observations were made. The 40 days old plants are exposed to Fluoride treatments *viz.*, 100, 200, 500 and 1000 ppm of fluoride contaminated water upto field capacity (1 liter) once a week for six weeks. The control seedlings were raised identically except without F in the irrigation water. Ten independent replications were made for each treatment.

Measurement of physiological responses

The infrared gas analyzer (IRGA-6400, portable photosynthesis system, USA) was used for monitoring photosynthetic CO_2 assimilation (P_N), stomatal conductance (g_s), and chlorophyll fluorescence yield (Fv/Fm) in fluoride irrigated cotton plants. These 84-day-old plants were used to obtain all physiological responses. Fv/Fm was measured using plant efficiency analyzer (PEA, Hansatech, UK) to assess the functionality of PS II to establish correlation with photosynthetic CO_2 assimilation.

Electrolyte leakage

Measurement of membrane leakage in leaf tissues was made by following Valentoric *et al.* (2006). The central portions of the leaf were cut into pieces of 1 cm, and 1 gm of these from each treatment was placed in test tubes containing 15 ml of deionized water. The tubes were then tightly capped and shaken for 24 hr at 25–30 ºC. The electrical conductivity (EC_1) of the solution was measured with an electrical conductivity meter (HI 8733, Hanna Instruments Inc., Woonsocket, USA). Afterward, the samples were autoclaved at 120ºC for 20 min and allowed to cool (25ºC) to record the electrical conductivity (EC_2) of the solution. The electrolyte leakage is expressed as,

$$\text{EL}\ (\%) = \frac{\text{EC}_1}{\text{EC}_2} \times 100$$

Shoot and root length, total leaf area and number

The leaf area expansion (cm^2), number and shoot and root length (cm) were measured after 50 days of F treatment. The leaf area was calculated by the paper-weight method with the help of a calibration curve. The harvest index was calculated by harvesting at least 10 seedlings of each treatment.

Fresh and Dry mass

The plants were removed from pots on 90 day and dipped in water filled bucket. The roots were washed carefully to remove the adhering soil particles. Fresh weight of roots and shoots was taken by digital single pan balance. The same plants parts were then placed in an oven at 70ºC till the weight become constant. The dried plant parts were weighed to record the dry mass of roots and shoots.

Average biomass production

The average fresh and dry biomass production/day/plant was measured as follows,

$$\text{Average biomass production (g/plant/day)} = \text{Biomass of 90 days old plant/90}$$

Harvest index

Harvest index (HI) was calculated as,

$$\text{HI} = \frac{\text{Economic yield (seed + cotton dry weight)}}{\text{Biological yield (leaf + stem dry weight)}}$$

Data (n=5) were analyzed by statistically by one way analysis of variance (SPSS, Statistical package and MS Excel) using Duncan's Multiple Range Tests (DMRT) to determine the significant differences among treatments at probability (p) 0.05.

RESULTS

Physiological responses

The effect of fluoride contaminated irrigation water on photosynthetic CO_2 assimilation (P_N), stomatal conductance (g_s) and chlorophyll fluorescence (Fv/Fm) variable yield per maximum yield was shown in figure 1(A, B & C) which was found to be decreased with increase the concentration of F in irrigatiuon water. The decrease in P_N, gs and Fv/fm was ~7–38, 8–38 and 4–27% respectively on the exposure of 100–1000ppm F as compare to their respective controls (Fig. 1. D, E & F).

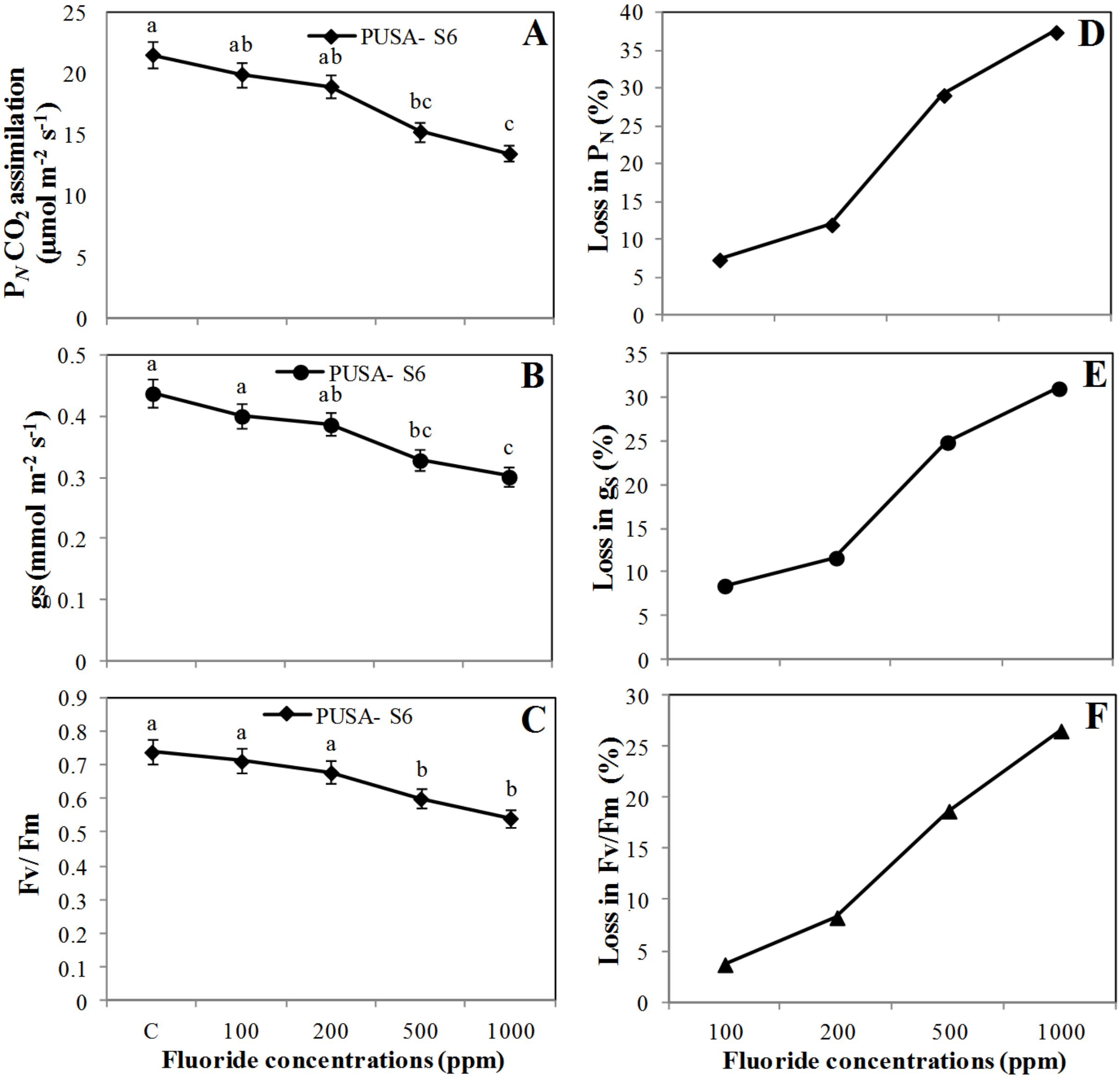

Figure 1. Effect of fluoride contaminated irrigation water on photosynthetic CO_2 assimilation (A), stomatal conductance (B) and chlorophyll fluorescence (Fv/Fm) variable per maximum yield (C) were as (D), (E) and (F) are the respective loss percentage. Observations were recorded with 4th leaf throughout by using 50 days old *G. hirsutum* plants after fluoride contaminated irrigation (n=6, weekly^{-1}). Each data point represents mean (n=5) with ±S.E.

Electrolyte leakage (EC)

Under the influence of fluoride toxicity the electrolyte leakage (EC) of leaf was affected very drastically. As the concentration of fluoride increased, the monotonic increase in EC was obsrved with significatdifferenceat p=0.05 (Table 1). The percent incecrease in EC at 100 ppm F concentratoin was 19%. Simillarly, the EC was increased 31, 51 and 67% on the exposure of 200, 500 and 1000 ppm F respectively.

Table 1. Effect of fluoride contaminated irrigation water on electrolyte leakage in cotton cultivar PUSA-S6. The observations were made after terminating the F treatment (n=6, weekly^{-1}). Each data point represents mean (n=5) with S.E. (±).

Treatments (ppm)	Leaf EC (%) PUSA-S6	Leaf EC (% increase) PUSA-S6
0	0	-
100	19	35.71
200	31	121.43
500	51	264.29
1000	63	350.00
Note: F value at p=0.05	212.486	

Shoot-root length, total leaf area and number

The phytotoxic effect of fluoride contaminated irrigation water upto 1000ppm was observed in the form of reduction in leaf area expansion (50% as compare to the control), leaf number (48.43%) and plant height. Interestingly, number of cotton ball was found highest in plants treated with 200 ppm F with improved harvest index (0.92). The cotton plant was found to be tolerant towards the exposure of fluoride in terms of yield of the crop. The effect of fluoride contaminated water irrigation on shoot and root length of cotton ciltivar (PUSA-S6) was represented in figure 2 (A & B). A finding of morphological (growth) experiment showed a decreasing trend in root length and shoots length with increasing sodium fluoride concentration. At 100 ppm F concentration, root and shoot length were ~4% less than that of respective controls. At 200ppm fluoride concentration, root and shoot length was decreased 12 and 18% respectively than of the respective controls. Similarly, at 500 and 1000 ppm F concentration root and shoot length was decreased very significantly (p0.05) as shown in figure 2 (C & D).

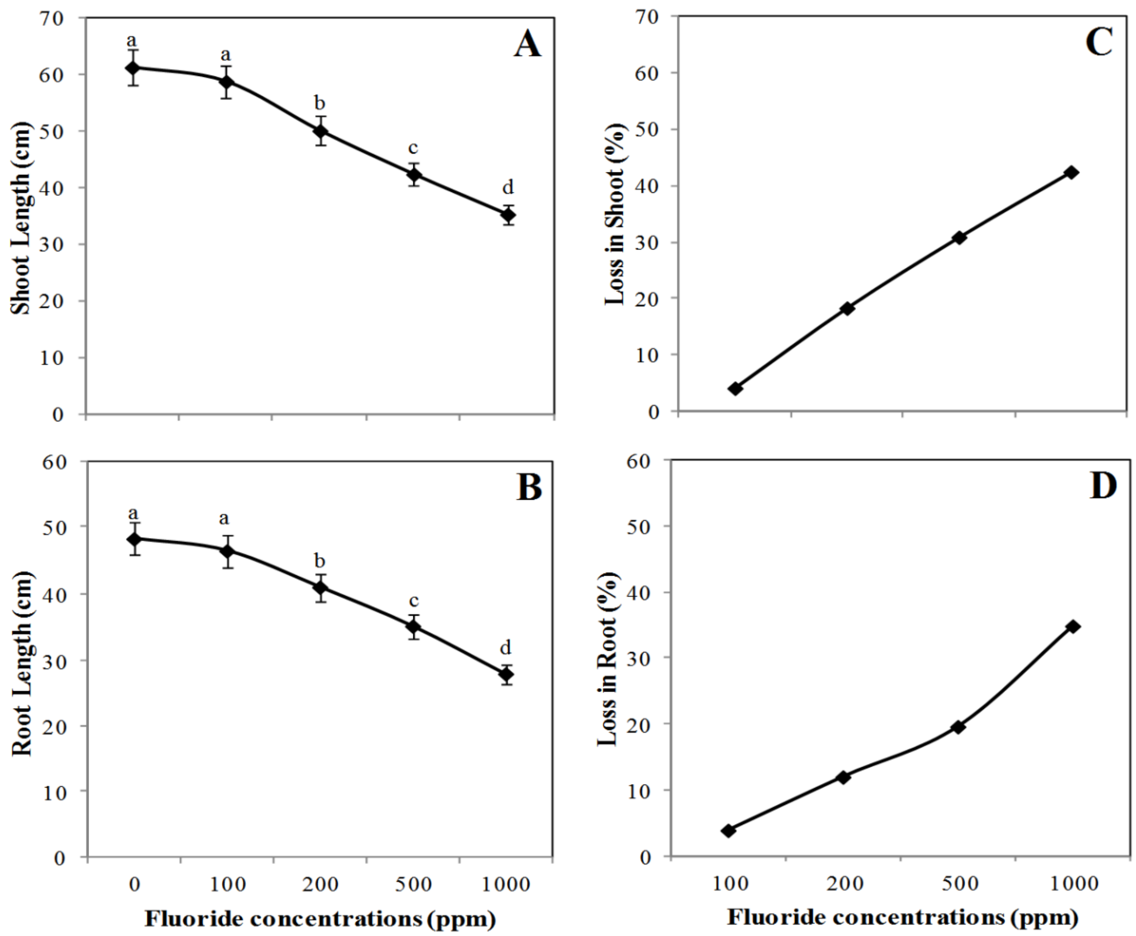

Figure 2. Effect of fluoride contaminated irrigation water on growth characteristics in *G. hirsutum*. The irrigation of contaminated water of various fluoride levels (n=6, weekly^{-1}). Each data point represents mean (n=5) with S.E. (±).

Fresh and Dry mass

The biomass of *G. hirsutum* was negetively correlated with the F treatments *i.e.*, significant decrease in shoot fresh and dry weight was observed. The decrease was reported to be maximum at 1000 ppm concentration of F as compared with their control. Decrease in stem fresh and dry mass at 1000 ppm was found to be 60.43 and 43.38% were as in root it was 61.70 and 73.18% (Fig. 3A–D) with respect to their coresponding controls.

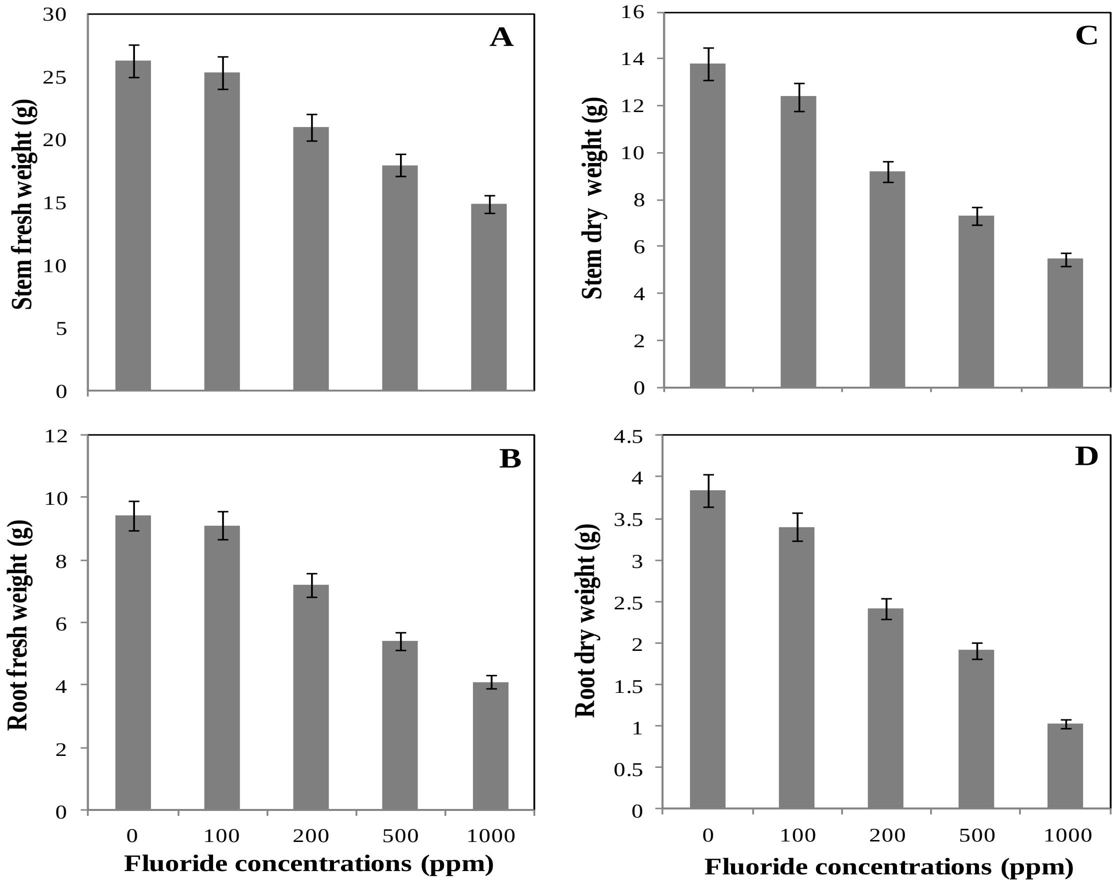

Figure 3. Effect of fluoride contaminated irrigation water on shoot and root fresh and dry mass of *G. hirsutum*. The irrigation of contaminated water of various fluoride levels (n=6, weekly^{-1}). Each data point represents mean (n=5) with S.E. (±).

Average biomass production

The average biomass production per day per plant was found to be decreased with incease the F concentrations (Table 2). The shoot average biomass production per day per plant was recorded to be 0.153, 0.138, 0.081 and 0.061 (g) with respect to the control, 100, 200, 500 and 1000 ppm respectively. The root average biomass production per day per plant was also found to be decreased under the infuence of increased F concentrations.

Table 2. Effect of fluoride contaminated irrigation water on shoot and root average dry mass accumulation of cotton cultivar PUSA-S6. The irrigation of contaminated water of various fluoride levels (n=6, weekly^{-1}). Each data point represents mean (n=5) with S.E. (±).

Treatments	Shoots DW/day/plant (mg) PUSA-S6	Roots DW/day/plant (mg) PUSA-S6
0	0.153 ± 0.0056^{a}	0.041 ± 0.0005^{a}
100	0.138 ± 0.0052^{b}	0.037 ± 0.0023^{b}
200	0.102 ± 0.0023^{c}	0.029 ± 0.0023^{c}
500	0.081 ± 0.0032^{d}	0.021 ± 0.0023^{d}
1000	0.061 ± 0.0017^{e}	0.012 ± 0.0017^{e}
F value at p=0.05	100.193	35.948

Harvest index

The harvest index of F irrigated plants favoured up-regulation (2–3%) for expressing plant productivity particularly economic yield (cotton/fibre-seed). The intrinsic physiological and biochemical membrane associated characteristics are yet to be revealed to diagnose possible logic of up-regulated plant productivity as influenced by fluoride contaminated irrigation levels (Fig. 4). The F toxicity affects the plant performance by reducing the shoot and root length. The harvest index showed the decreasing trend with increasing concentration of F.

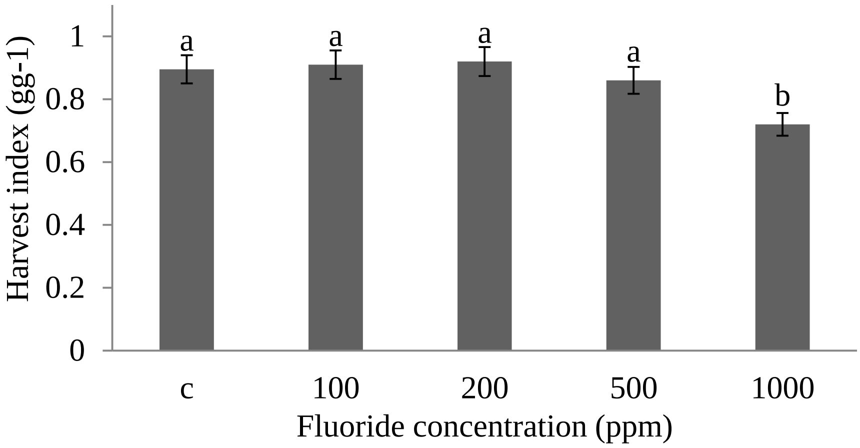

Figure 4. Effect of F contaminated water irrigation on harvest index in *G. hirsutum*. Each data point represents mean (n=6) with ±S.E.

The morphological and growth pattern of the plant irrigated with F contaminated water is depicted in figure 5. The plants showed decrease in height, leaf number and leaf area. It may be due to the phytotoxic effect of F. At 100 ppm F concentration, the plant height was 8.22% less than that of control. At 200 ppm it was 16.43% were as at 500 ppm it was 24.65% and finaly at 1000 ppm the loss in plant height was measured 32.88% than that of control. Here the plants are arranged in order of fluoride treatment, *i.e.* C, 100, 200, 500 and 1000 ppm.

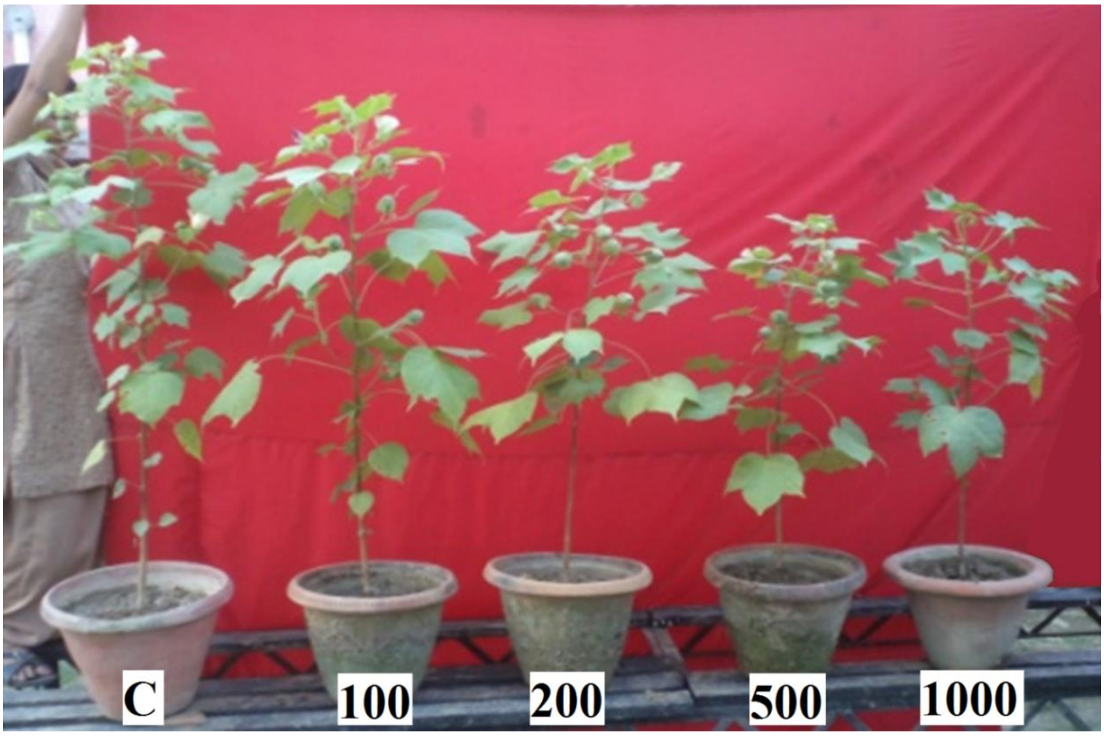

Figure 5. Effect of fluoride contaminated irrigation water on morphology of *G. hirsutum* plants. The photographs were obained after terminating the treatment (50 days after application of fluoride contaminated water, n=6, weekly^{-1}). The control plants (C) were irrigated with normal tap water with treatments viz., 100, 200, 500 and 1000 ppm.

DISCUSSION

Fluoride is a common pollutant in water and soil that inhibits seed germination, causes ultra-structural malformation of chloroplast and mitochondria and alters membrane permeability with impaired photosynthetic efficiency thereby affecting plant growth, productivity and biomass (Rubio-Casal *et al.* 2003, Elloumi *et al.* 2005, Sabal *et al.* 2006, Gupta *et al.* 2009, Gautam & Bhardwaj 2010, Datta *et al.* 2012, Singh & Verma 2013). Due to bioaccumulation of F in the roots of the plants Zhang *et al.* (2013), it enters into the most efficient photosynthetic zone, *i.e.* mesophyll cells, thereby resulting in loss of chlorophyll fluorescence yield (Fv/Fm) of PS II. The reduction in photosynthetic CO_2 assimilation is correlated with impaired CO_2 diffusion (Karolewski *et al.* 2000, Walna *et al.* 2007) and light harvesting chlorophyll pigments (Kamaluddin & Zwiazek, 2003, Pant *et al.* 2008, Chakrabarti *et al.* 2013). It is also reported that NaF, presumebly block the dephosphorylation of membrane protein (particularly the chlorophyll a/b protein complex) which is phosphorylated by light-activated kinase. Thus fluoride facilitates the attainment of state 2 by interfering in state 1 to 2 transition Canaani *et al.* (1984). And also the change in the PS-I / PS-II ratio have mostly assosiated with the rearrangment of chlorophyll proein complexes in side thalakoid membrane Garstka *et al.* (2005).

The fluoride stress affects the plant physiology and growth adversely with increase in concentration of F contamination irrigation water *i.e.* 100, 200, 500 and 1000ppm, the toxicity level increases which results the decrease in biomass content of the plant (Fig. 2, 3). The fluoride affects photosynthesis by affecting the membrane permeability–integrity and associated enzymes of Calvin cycle essential for carboxylation of atmospheric CO_2 in chloroplast (Garrec *et al.* 1981, Gautam & Bhardwaj 2010). The higher values of the fluoride-contaminated irrigation water have also impaired physiological responses, *i.e.*, P_N, g_s and Fv/Fm (Fig. 1) and also affected plant performance, productivity and biomass (Qingtao *et al.* 2002, Gautam *et al.* 2010, Verma 2011, Singh & Verma 2013). The cumulative effect of above resulted in the reduction of leaf area expansion and leaf number with depressed chlorophyll biosynthesis and its organization in the younger leaves. With increased F concentration the leaf area expansion, leaf number was monotonically reduced. Thus, loss in leaf area (Kamaluddin & Zwiazek 2003, Doley 1986, 2010) chlorosis and necrosis reduced canopy photosynthesis (Doley 2012).

Under stress condition plants membranes are subjected to changes such as increase in permeability and loss of integrity Blokhina *et al.* (2003). The degree of cell membrane injury induced by stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji *et al.* 2002). Higher electrolyte leakage indicates lower membrane stability index of plant cell. At lower F concentration, less electrolyte leakage was observed. Hence, an increased electrolyte leakage with enhanced exposure to F, thereby apparently impairing structural integrity of the biomembrane with loss in photosynthetic CO_2 assimilation (Zwiazek & Skay 1988a). From the following it can be concluded that fluoride concentrations were inversly proportional to the membrane intigity.

Fluoride causes reduction in root and shoots length due to unbalanced nutrient uptake by seedlings in presence of fluoride (Sabal *et al.* 2006). Reduction in root length and shoot length has also been reported by various researchers in *Pisum sativum* (Sabal *et al.* 2006, Prachi 2012), Cluster bean (Sabal *et al.* 2006). *Brassica juncea* (Pant *et al.* 2008), *Oryza sativa* (Elluomi *et al.* 2005), *Triticum aestivum* (Bhargava & Bhardwaj 2010) and *Cicer arietinum* (Dutta *et al.* 2012).

Fluoride causes reduction in root length and shoot length due to unbalanced nutrient uptake by seedlings in presence of fluoride (Sabal *et al.* 2006). Fresh weight and dry weight decreased monotonically with increasing fluoride concentration due to reduction of metabolic activity in presence of fluoride because germination is a one kind of metabolism and fluoride acts as a metabolic inhibitor (Gulzar & Khan 2001, Gupta *et al.* 2009, Sabal *et al.* 2006).

A significant elevation in activities of peroxidase, catalase, and ascorbic acid oxidase is also found to be dependent on severity and duration of fluoride exposure (Karolewski *et al.* 2000, Walna *et al.* 2007, Kumar *et al.* 2009) which may affect the quality and quantity of the plant produce. Thus, as seen in figure 1, 2, 3 and 5, after F irrigation, loss in leaf area-expansion, leaf number, shoot length, chlorophyll, PS II efficiency, stomatal conductance, and CO_2 assimilation occurred with enhanced electrolyte leakage in the leaf cells, leading to a reduction in harvest index (HI).

CONCLUSION

Fluoride affect adversely to both animals and plants. But in this research it was found that, the cotton can resist fluoride up to 200ppm in pot culture because the harvest index was slightly increased at this concentration. Therefore, the cotton cultivation may be extended in F-affected (~200 ppm F) agro-climatic zones to sustain the agro-socio economy.

ACKNOWLEDGEMENTS

This research was made possible by support from the University Grant Commission (UGC), New Delhi, by an award of Rajiv Gandhi National Fellowship (RGNF) to Mr. S. Kumar and it is gratefully acknowledged.

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