Indian J. Applied & Pure Bio. Vol. 38(1), 114-124 (2023). **ISSN: 0970-2091**
A web of Science Journal

Examining the effect of Calcium on Fluoride toxicity of fenugreek (*Trigonella foenum-graecum* L.) growth

Jinita J. Gamit[1] and J.S. Patel[2]

Department of Botany, The HNSB Ltd Science College,
Himatnagar- 383001 (India)
Email id- 1. gamitjinita@gmail.com
2. drjsp@gmail.com

Abstract

The aim of the study is to examine the effect of calcium on fluoride toxicity on fenugreek plant. Fluoride toxicity is a common problem in India. Fluoride has both kind of effect- beneficial and harmful. Calcium in diet or given to plant directly or indirectly can reduce the fluoride toxicity. NaF and NaF with $CaCl_2$ was taken as soil pollutant. The 10 seeds were sown in each pot and necessary watering was done and vegetative data e.g. length of root and shoot, no. of leaves, fresh and dry weight of root, shoot, leaves and reproductive growth data also were taken at the interval of every 15 days up to the yield. As a result, significant reduction was observed in length, fresh and dry weight of plants containing only NaF. Where $CaCl_2$ was mixed with fluoride showed significant toxic effect of fluoride is decrease in compared to fluoride containing fenugreek.

Key words : Calcium, Fenugreek, Fluoride, Growth, Toxicity.

India is one of the largest producer, consumer and exporter of spices. India is known for its food flavours all over the world and known as "Land of spice" (Wikipedia). It is the major producer of fenugreek (*Trigonella foenum-graecum* L.) and its main consumer for culinary and medicinal uses. Fenugreek is one of the oldest medicinal plant originating in India and North America[5]. The major fenugreek producing states are Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Maharashtra and Punjab (Wikipedia). It has a long history of medicinal uses in Ayurveda and in Chinese medicine. It is well known for its fiber, gum, other chemical constituents and volatile contents. There are several reports concerning the anti-inflammatory and antipyretic effect of the plant.

Fluoride is wide spread in nature and is estimated to be thirteenth in abundance among the elements of the earth[12]. Fluoride is not an essential element for human development except bone and teeth development. When it is given in low concentration it cannot be harmful but if excessive amount of fluoride given for a long period may cause detrimental

effect on living organism. Most of the food whether they are derived from plants or animals contains minute amount of fluoride. Intake of fluoride into plant is purely dependent on the type and concentration of fluoride ion in the soil[2,8]. Wheat germ grown in an area of North Africa with an unusually high fluoride ion content 7470 ppm in soil contained 2 times more fluoride ion that wheat germ in France. If fluoride is present in acidic soil, its uptake by plants increased. Liming soil with calcium and addition of phosphates can reduces fluoride ion intake by plants[7]. The pooled concentration of fluoride in India is around 2.37 mg/L with 95 % confident level. The states with a high prevalence rate of fluoride in India are Assam, Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Haryana, Jharkhand, Karnataka, Jammu and Kashmir, Maharashtra, Punjab, Rajasthan, Tamil Nadu and New Delhi. According to department of drinking water supply, ministry of rural development fluoride is the second major contaminant in India.

Pharmacologically significant is its strong affinity for calcium and other metal with which it forms highly complex compound. Some of its action has been ascribed to its calciprive effects[7]. Calcium is an essential element, however its role is elusive. Many studies have shown that the reduce fluoride uptake followed by calcium application appeared not to be due to simply to the precipitation of CaF_2 and soil or to the complexing of Ca and F in roots. It was more likely due to the effect of Ca on the properties of cell wall or membrane permeability in the solution experiment (Wikipedia). It has been observed that the uptake of calcium is inhibited by fluoride, hence the complexing of fluoride with calcium has been proposed as a mechanism underlying F toxicity in plants.

Material : - NaF, $CaCl_2$, Fenugreek seed, Weighing scale, Polythene bag, Soil, vermicompost.

Methodology : - Dry and healthy seeds of fenugreek was taken. This experiment was conducted in winter season. First of all, 5 kg pre-weighted soil is filled in polythene bag. Vermicompost is used as manure. For this experiment two sets of polythene bag were made. In this experiment fluoride was taken as soil pollutant. In one polythene only soil and vermicompost was mixed and this is used as control condition (P1) which was irrigated by distilled water and in other polythene bag different concentration of fluoride was taken *e.g.* 100 ppm NaF (P2), 200 ppm NaF (P3), 300 ppm NaF (P4), 400 ppm NaF (P5), 500 ppm NaF (P6) was added in 5 kg pre-weighted soil. In second set $CaCl_2$ was mixed with NaF as the same concentration as first set *e.g.* 100 ppm NaF + $CaCl_2$ (P7), 200 ppm NaF + $CaCl_2$ (P8), 300 ppm NaF + $CaCl_2$ (P9), 400 ppm NaF + $CaCl_2$ (P10), 500 ppm NaF + $CaCl_2$ (P11). 500 mg vermicompost was added to all the bags. Necessary watering was done at the interval of every 2 days. Data were taken after every 15 days up to the life cycle. Data *e.g.* length of root and shoot, number of leaves, no. of flowers and pods and fresh and dry weight of root, shoot ,leaves and pods were measured.

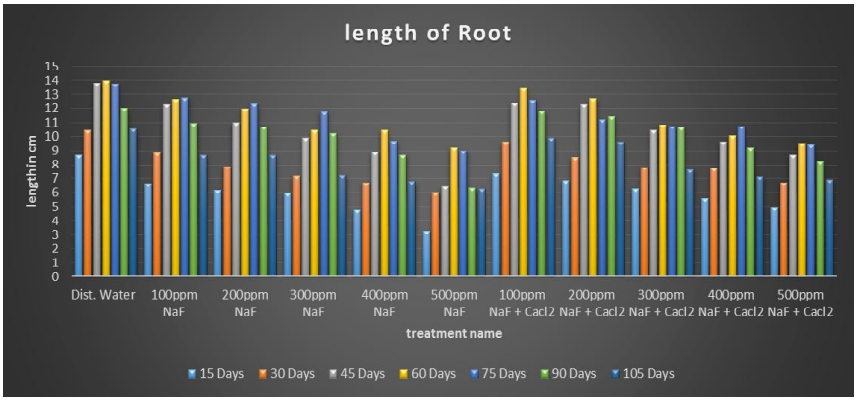
Vegetative growth :

As seen in the graph no. 1 from the set 1, the root length, which were treated with

only fluoride, shows suppressed growth as the concentration of fluoride increases. In some research done on *Triticum aestivum* shows that accumulation of fluoride in root, shoot, leaves and seed shows monotomic trend with increasing concentration of fluoride in irrigation water[1].

In the first set, root length is not much affected in P2 (100 ppm NaF) & P3 (200 ppm NaF) lower concentration but as the concentration increases P4 (300 ppm NaF), P5 (400 ppm NaF) and P6 (500 ppm NaF) root length decreases over time. Earlier study have shown that severe impact on the growth of tomato species grown in a solution culture containing fluoride concentrations above 260 µm[11]. At the higher concentration 400 ppm NaF (P5) and 500 ppm NaF (P6) and root and shoot length shows the shortest length. In the second set in which calcium was mixed with fluoride shows significant increase over the NaF series plant. From the graph no. 2 same as the first set of experiment P7 (100 ppm NaF with $CaCl_2$) and P8 (200 ppm NaF with $CaCl_2$) shows normal growth at some point. Higher concentration 300 ppm NaF + $CaCl_2$ (P9), 400 ppm NaF + $CaCl_2$ (P10), and 500 ppm NaF + $CaCl_2$ (P11) shows relatively short root length than lower concentration but this root length is higher than Only NaF treated plant. The second set (NaF + $CaCl_2$) compare with NaF plant series, root length shows increase over NaF plant.

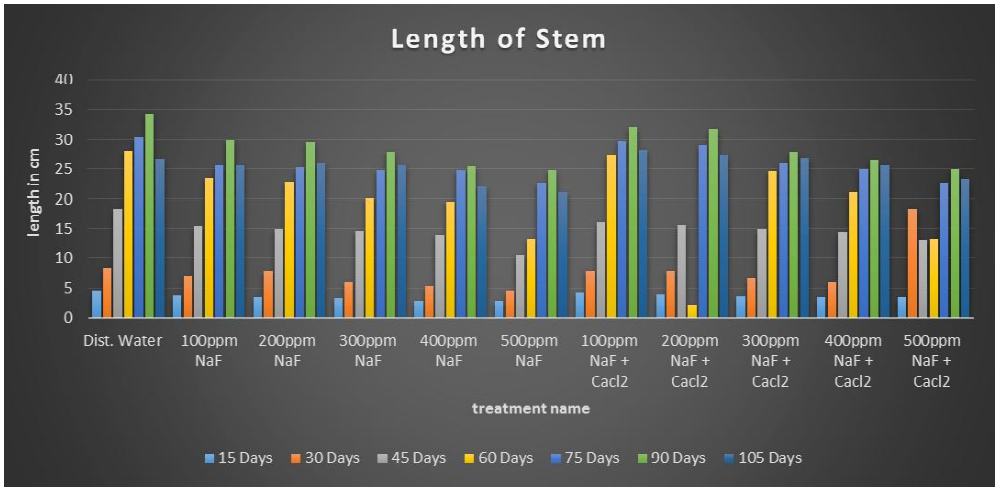
Same as the root length, shoot length also shows same result. Higher concentration of fluoride decreased the shoot length in first set of experiment while in second set the length of shoot shows better length over only NaF treated plants. In the control condition treated plant P (1), the length of the root and shoot shows higher growth than that of both set of experiment. . In a study with *vicia faba* the ability of roots to accumulate higher amount of F than that of the shoot system was noted. Very high uptake and accumulation of F have been recorded in the range of 280 to 4000 mg/ kg for both grass and legume species[11]. From the graph no. 03, we can say that number of leaves decreases as the concentration of fluoride increase but at the low concentration *e.g.* 100 ppm NaF P (2) and 200 ppm NaF P (3) not much affected by fluoride but at higher



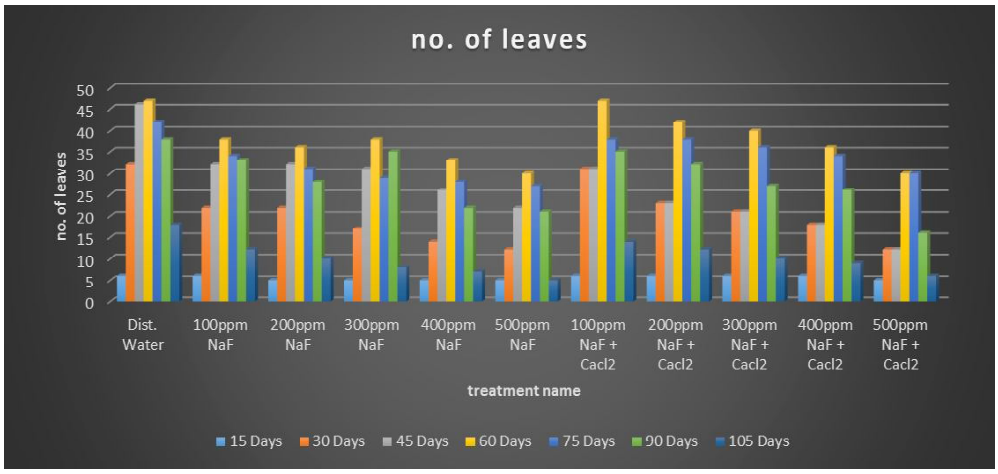
Graph no: - 01 Effect of NaF + NaF and $CaCl_2$ on root length of fenugreek.

concentration 300ppm NaF P (4), 400 ppm NaF P (5) and 500 ppm NaF (P6), leaf margin shows necrosis, where there was no necrosis and chlorosis of leaves, a reduction in the in the irrigation water caused necrosis and chlorosis of leaves, a reduction in the growth of the shoot and root and yield of mustard[2].

Leaves of the plants kept as control were completely green and healthy and no trace of necrosis was seen. and the no. of leaves was higher than the symptoms of leaf margin necrosis seen in the second set series which was mixture of calcium and fluoride.



Graph no: 02 Effect of NaF and NaF + $CaCl_2$ on the length of stem of Fenugreek



Graph no:- 03 Effect of NaF and NaF + $CaCl_2$ on the no. of leaves of fenugreek

In the present study, high concentrations of fluoride in the irrigation water caused. Plants which were treated with calcium and sodium fluoride and only sodium fluoride treated plant.

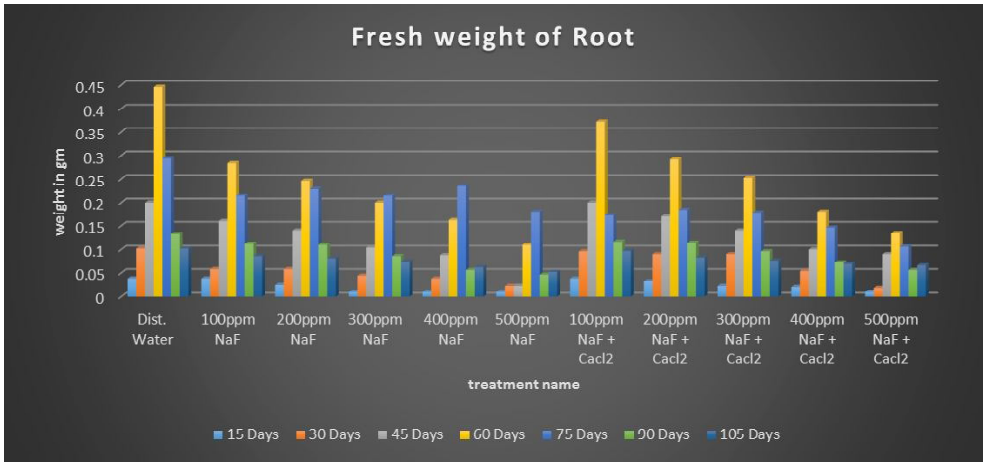
Fresh weight :

As seen from graph no. 4, 5 and 6, Leaf contains the higher fresh weight followed by stem and root. Control condition (P1) shows the highest weight among the experiment. Lower concentration from the set 1 experiment, 100 ppm NaF (P2), 200 ppm NaF (P3) shows normal and not much affected growth but as the concentration of fluoride increases 300ppm NaF (P4), 400 ppm NaF (P5) and 500 ppm NaF (P6) shows less fresh Weight. In the second set calcium and fluoride treated plants shows more weight than that of only fluoride containing plants but less than control condition.

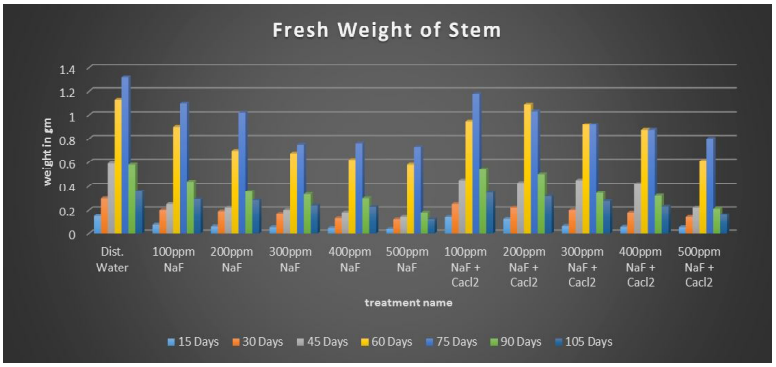
Fresh weigh of stem in the control condition has the higher fresh weight followed by fluoride and calcium series P7 to P11. In the series of only fluoride containing plant P3 to P6 shows gradually decreasing weight but at the higher concentration P6 has the lowest fresh weight among all the series due to excessive fluoride. In the second set of experiment in which calcium was added with fluoride has the relatively high fresh weight of root and shoot (graph no. 4 & 5) than only fluoride containing plant. Lower concentration of calcium and fluoride series 100 ppm NaF+ $CacCl_2$ (P7) and 200 ppm Stunt fresh weight but 500 ppm NaF+$CaCl_2$ (P11) shows relatively better fresh weight than 500 ppm NaF.

Dry weight :

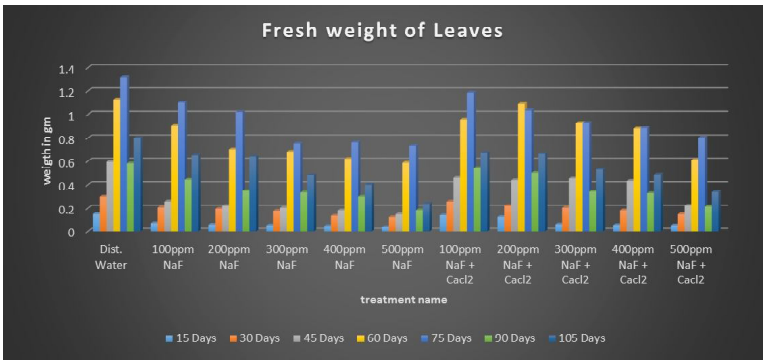
Fenugreek plant shows relatively low dry weight of plant parts (Graph no 7, 8 & 9). Same as the fresh weight, fluoride treated plants shows stunt dry weight due to NaF+ $CaCl_2$ (P8) shows the normal fresh weight but more weight than100ppm (P2) and 200ppm NaF (P3).



Graph no: - 04 Effect of NaF and NaF+ $CaCl_2$ on the fresh weight of root of fenugreek



Graph no: - 05 Effect of NaF and NaF+ $CaCl_2$ on the fresh weight of stem of fenugreek



Graph no: - 06 Effect of NaF and NaF $CaCl_2$ on the fresh weight of leaves of fenugreek

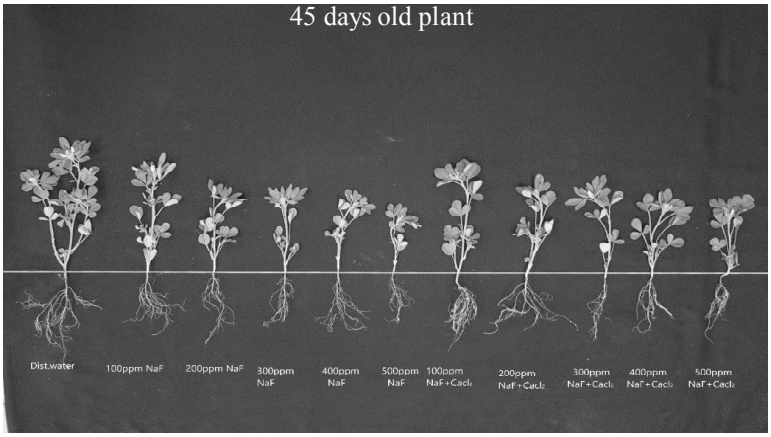
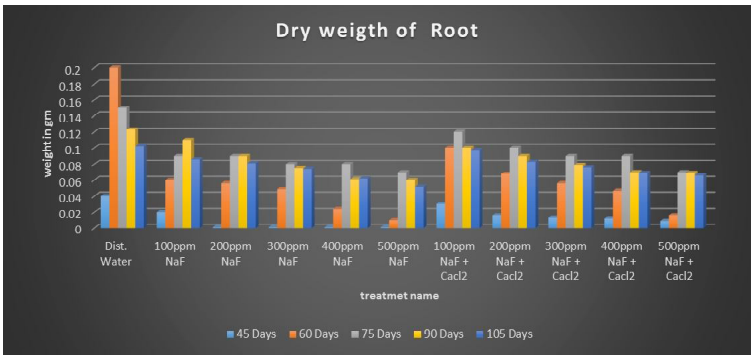
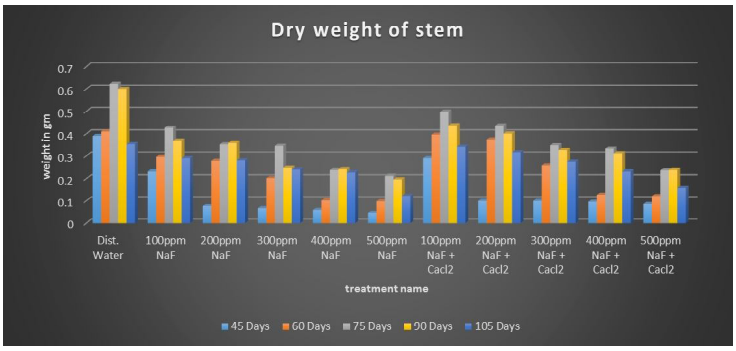


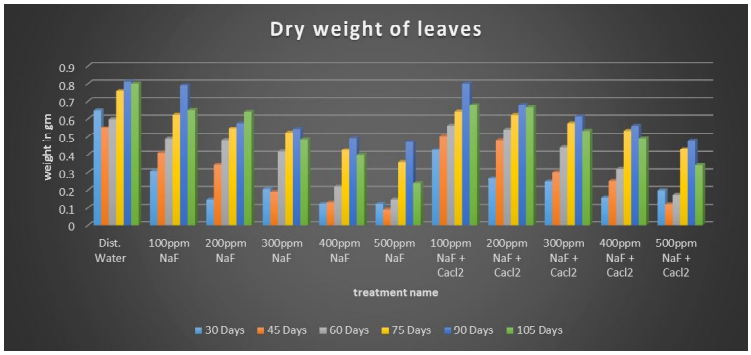
Fig. 1. Effect of NaF and Na+ $CaCl_2$ on fenugreek plant on 45th day



Graph no: - 07 Effect of NaF and NaF + $CaCl_2$ on the dry weight of root of fenugreek plant



Graph no: - 08 Effect of NaF and NaF + $CaCl_2$ on the dry weight of stem of fenugreek plant



Graph no: - 09 Effect of NaF and NaF + $CaCl_2$ on the dry weight of leaves of fenugreek plant

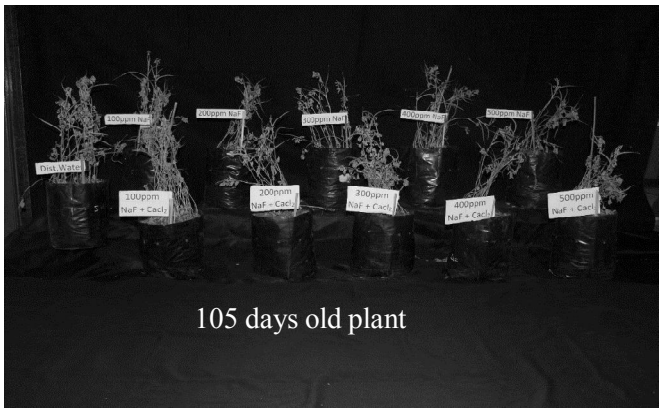


Fig. 02 Effect of NaF and NaF + $CaCl_2$ on fenugreek plant on 105th day (yield plant)

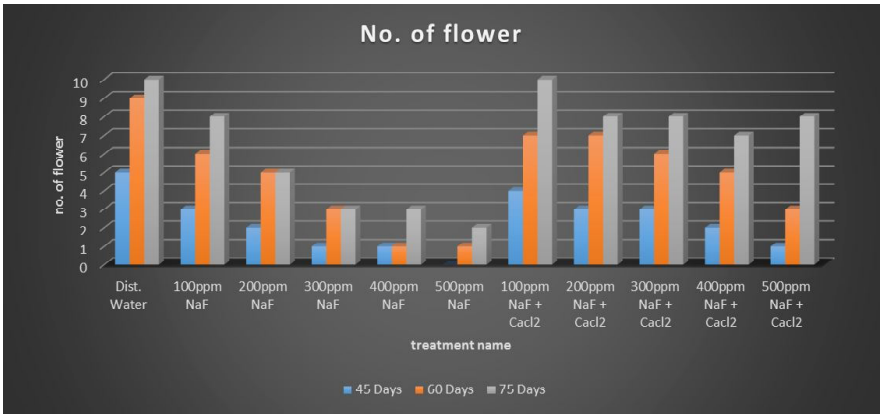
Higher concentration 500 ppm NaF (P6) shows the excessive fluoride and calcium make it less severe which is seen in second set P7 to P11 series plant.

Reproductive Growth :

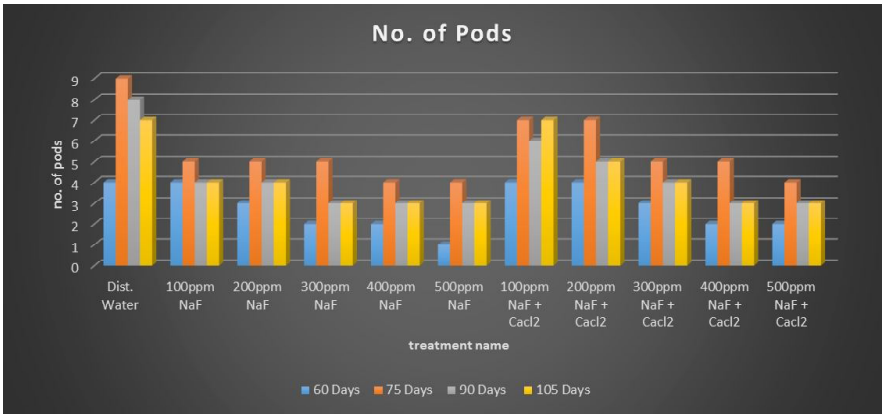
As seen in graph no.10 after the 28 days of seed sown, the flower start blooming in the control condition fenugreek plant. Only one flower seen in 100 ppm NaF+ $CaCl_2$ (P7) treated plant. After the 42 days old plant flower has bloom in all the treatment except the higher concentration of NaF 500 ppm NaF (P6).

In 500 NaF+ $CaCl_2$ (P6) first flower appear after 56 days. At Lower concentration P4, P5 and P11 has the same no. of flower and P8 and P9 has the same no. of flowers.

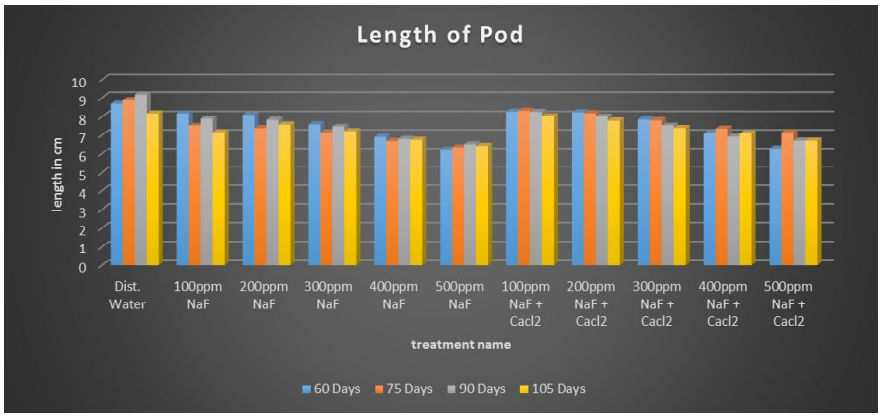
First pod appear in control condition after 48 days. In the first set of experiment, NaF treated plants 100 ppm NaF (P2) and in second set treatment 100ppm NaF + $CaCl_2$ (P7) and 200 ppm NaF $CaCl_2$ (P8) has the same no. of pods as the control condition. 500 ppm NaF (P6) has the lowest number of pods.



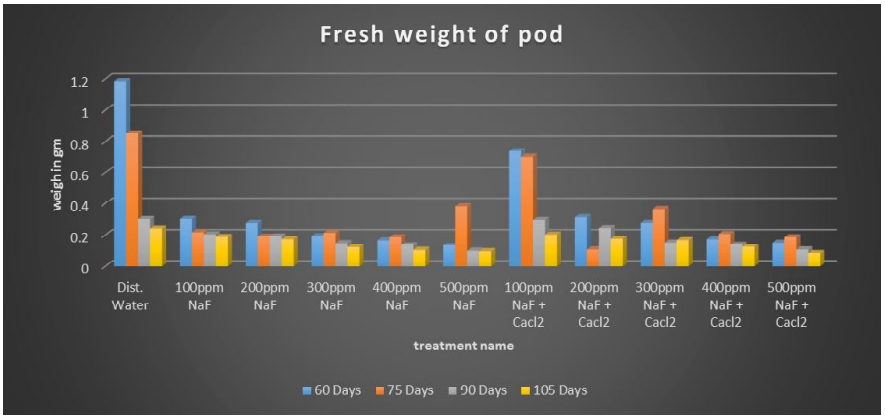
Graph no:-10 Effect of NaF and NaF+ $CaCl_2$ on no. of flower in fenugreek plant



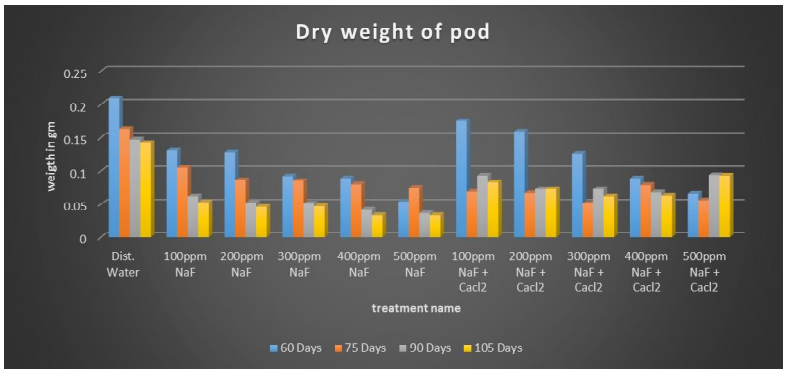
Graph no:-11 Effect of NaF and NaF + $CaCl_2$ on number of pods in fenugreek plant



Graph no:-12 Effect of NaF and NaF + $CaCl_2$ on length of pods in fenugreek plant



Graph no:-13 Effect of NaF and NaF+ $CaCl_2$ on fresh weight of pods



Graph no:-14 Effect of NaF and NaF + $CaCl_2$ on dry weight of pods in fenugreek

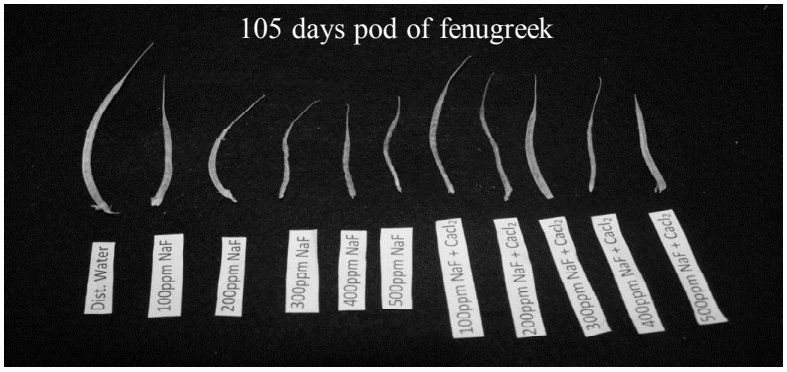


Fig. 3 Effect of NaF and NaF + $CaCl_2$ on pods of fenugreek (Dry)

The length of pod also reduce as the no. of pods reduce in the experiments. Length of pods were higher in control condition followed by Calcium and fluoride treated plant pod (P7 to P11) and then only fluoride containing plant pod (P2 to P6).

As seen in root and shoot fresh and dry weight, same results were seen in the fresh weight of pod and dry weight of pod. Control condition pods has the maximum fresh weight and dry weight than other series plant pod. In the second set plants (calcium and fluoride treated plants) shows better growth than only fluoride treated plants.

The lowest concentration of fluoride plants. The lowest concentration of fluoride 100 ppm NaF and 200 ppm NaF (P2 and P3) shows better fresh and dry weight than higher concentration 300 ppm NaF, 400 ppm NaF and 500 ppm NaF (P4, P5 and P6).

So, the accumulation of fluoride at the pre stage was maximum in the leaves and minimum in the stem and root[1]. So from this result we can say that the control condition plant (P1) has overall the healthy growth which is continuously followed by second set of experiment (P7 to P11). And from P5 & P6

of experiment shows stunt growth which was treated with only fluoride. Where 100 ppm NaF (P2) & 200 ppm NaF (P3) & 300 ppm NaF (P4) experiment series shows somehow normal growth but it was slightly affected by fluoride toxicity. In the second set where calcium was mixed showed better growth even in higher concentration 500 ppm NaF + $CaCl_2$ (P11) series.

From this series it can be easily observed that adding calcium in P7 to P11 series reduce the fluoride toxicity to some extent. Burkhart and jowsey showed that a high oral intake of calcium by fluoride fed kittens prevented the development of the osteoporosis which resulted from fluoride and a low calcium intake and also produced bine which was morphologically normal[9]. So from this one can concluded that calcium can make less sivere effect of excessive fluoride.

References :

1. Agarwal Rinku, and Surendra Singh Chauhan. (2014). *International Journal of food, agriculture and veterinary sciences*. *4*(1): 98-101.
2. Ahmad Shakil, S. Saeed Ahmad, M. Nauman Ahmad, R Ullah Shah, and Muhammad Nawaz, (2015). *Research report fluoride 48*(2): 169-173.
3. Dey Uttiya, Naba Kumar Mondal, Kousik Das, and Jayanta Kumar Datta (2012). *Research report fluoride 45*(4): 389-393.
4. Diamanti, I., H. Koletsi-Kounari, E. Mamai-Homata, and G.Vougioklakis. (2010). *Journal of Dentistry, 38:* 671-677.
5. Ethan basch, Catherine Ulbricht, Grace Kuo, Philippe szapary, and Michael smith (2003), *Alternative Medicine Review*, *8*(1): 20-27.
6. Fan, Y., Z. Sun, and J. Moradian Oldak (2009) *Caries Research, 43:* 132-136.
7. Geoege Waldbott, (1963), *The American journal of clinical nutrition 12*(6): 455-456.
8. Hong Byeong Deok, Ri Na Joo, Kyo suk lee, Dong sung lee, Ja- hyun Rhie, Se won min, Seung-Geun song, and Doug- young chung (2016). *Journal of Agricultural science*, *43*(4): 522-536.
9. Jowsey, Jenifer, B. Lawrence Riggs, Patrick J. Kelly, and David L. Hoffman (1972). *The American Journal of Medicine*, *53:* 43-49.
10. Kassie Nigus, Bhagwan Singh Chandeavanshi, and Addis Ababa (2016). *Research report Fluoride 49*(2): 165-177.
11. Pant Sudhakar, Prabhakar Pant, and P.V. Bhiravamurthy. (2008). *Research report Fluoride. 41*(1): 57-60.
12. Parikh Punita, and Rahul Parikh (2015). *International journal of Allied practice, research and review. International peer, reviewed referred journal 2*(1): 50-54.
13. Srivastava, Anushree, Madhu Kumari, Kumar Suranjit Prasad (May 2020). *Indian journal of chemical technology, 27:* 248-253.
14. Wang Jung dong, yuhong guo, Zhab Xue liang, and Junhy Hao, Shanxi (2003). *Fluoride*, *36*(3): 177-18.