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RESEARCH ARTICLE

Effect of Salt Stress on Germination and Growth of *T. foenumgraecum* Seedlings.

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Abstract

Seedling establishment is a critical stage that determines production of crop at later stages, thus it necessitates studying the impact of salt stress at primary level. Present study is aimed to evaluate the effect of salinity stress on germination as well as growth of seedlings of *T. foenumgraecum*. Seedling germination showed 100% germination of seeds upto 100mM NaCl treatment but beyond it there is a sharp decline in germination percentage as it showed 60% decline in 200mM NaCl added set. Salt stress at higher salinity levels (beyond 100mM NaCl) also resulted in delayed seedling germination. Results showed there is not much variation in 50 and 100mM NaCl treatments in comparison to control. But it shows strong inhibition in terms of length as well as fresh weight of different plant parts as cotyledon, hypocotyls as well as roots of seedlings with increasing level of salt solution particularly at higher salt treatments as 150 and 200mM NaCl treatments. This decline was more prominent in roots (71.43% and 73.47%) then in shoots (54.8% and 55.06%) and least in cotyledons (21.87% and 19.51%) in comparison to control in 7 and 15 days old fenugreek seedlings. The increasing salt concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices and the seedling vigor indices.

Introduction:-

This is a well known fact that Salinity is a complex environmental constraint and one of the major abiotic stresses present in irrigation water that is affecting the growth as well as yield of crops. Over 25% of soil area in world is saline which limits the growth and production of the plant. Under the effect of salinity, pH of soil exceeds from 8.5 and electrical conductivity more than 4 dSm-1 which cause a reduction in productivity of the plant (Ashraf, 1994). Seed has a role in the initiation of next generation. The embryo present in the seed acts as a miniature plant and provides food reserve to the growing seedlings. Seed germination and early seedling growth are critical events for plant development (Kitajima and Fenner, 2000) and crop production (Almansouri *et al*., 2001). This salinity impairs seed germination, seedling growth and in later stages productivity of plants. The genetic potential for salt tolerance is indicated by the ability of a seed to germinate under salt stress (Tejovathi *et al*., 1988). Developing varieties with greater salt tolerance can improve productivity. A better understanding of the dominant effects involved in plant responses to salinity will facilitate development both of improved varieties and crop management practices. Therefore, the objective of the present study was to evaluate the effects of salinity on fenugreek in terms of germination and seedling growth, their sensitivity to salt stress, in order to better understand the mechanism of salt tolerance in this medicinal plant. In this study, we aimed to determine the effects of salinity levels (0, 50, 100, 150 and 200 mM) on germination and seedling growth of fenugreek.

Materials and Methods:-

Seeds of *T. foenumgraecum* were procured from local market of Meerut. Seeds were hand sorted to eliminate broken and small seeds. Uniformly selected seeds were sterilized with 0.1% mercuric chloride for 1 min and then washed repeatedly for two to three times under running tap water followed by washing with distilled water. Seeds were then germinated in glass Petri dishes of 100 x15 mm diameter. The glassware prior to use was thoroughly washed with tap water followed by a rinsing with distilled water and then sterilized at 170^{o}C for 4 hours. The dishes were incubated at 25^{o}C. Seeds were placed on twice folded Whatman # 1 filter paper seed beds soaked in a solution of the respective salt concentration (Ctrl, 50, 100, 150 and 200mM NaCl) in Petri dishes. Each Petri dish was provided with 5 ml of the respective salt concentration. The seed germination was evaluated after every 12 h. Different physical parameters were studied. Seed germination started after 24 h (seeds were considered to be germinated with the emergence of the radical). The germinating seeds were counted at daily intervals. The lengths of root and shoot of the germination seeds which were >2 mm in length were measured and recorded upto 15 days after radical emergence. Seedlings from each of the treatments were measured for fresh and dry weight determination. After 7 and 15 days after radical emergence, plant seedlings were removed carefully from the Petri plates and separated into shoots and roots. After recording fresh weights, the plant samples were oven-dried at 65^{o}C for 72 hours and their dry weights measured. The moisture contents of seedlings were determined on oven dry basis (Hussain, 1989).

Seed germination studies:-

- Seed germination
- Rate of Germination
- Germination percent (GP) = $\frac{\text{Number of germinated seeds at final count}}{\text{Total number of seeds sets for bioassay}} \times 100$
- Germination index (GI) = $\Sigma GT/Tt$ Or
- $$= \left(\frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{Number of germinated seeds}}{\text{Days of last or final count}} \right)$$

Seedling Parameters**:-**

- Shoot length (cm) Root length (cm)
- Fresh matter (g) Dry matter (g)
- Root/Shoot ratio
- The Seedling Vigor Index (SVI) **Iqbal and Rahmati (1992)**
  VI = (mean root length + mean hypocotyls/shoot length) X Percent germination
- Phytotoxicity for shoot and root of seedlings **Chou and Lin (1976)**:
  $\% \text{ Phytotoxicity} = \frac{\text{length of control - length of treatment}}{\text{length of control}} \times 100$
- Stress tolerance index is a useful tool for determining the high yield and stress tolerance potential of genotypes. **Iqbal and Rahmati (1992)**

T.I. = (Mean length in salt solution / Mean length in distilled water) x 100%
RLSTI = Root length Stress Tolerance Index
SLSTI = Shoot length Stress Tolerance Index
RFSTI = Root fresh weight Stress Tolerance Index
SFSTI = Shoot fresh weight Stress Tolerance Index

Each treatment was replicated thrice and the data represent average values. All the chemicals used were of analytical grade reagent (Merck India, SRL).

Results and Discussion:-

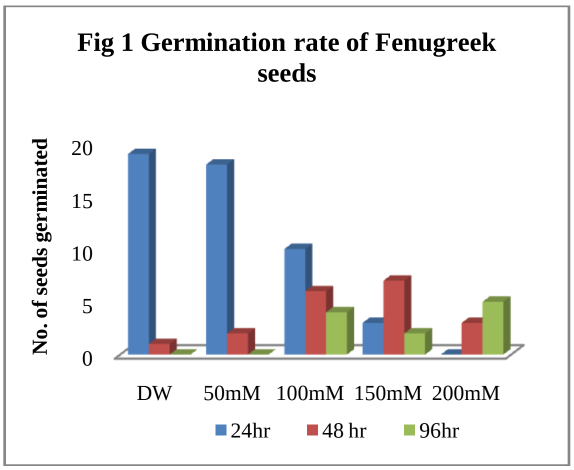
Table-1 viability test in trigonella seeds

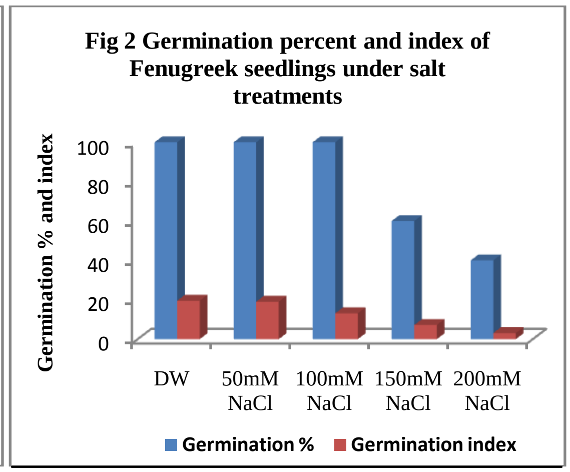
S.No.	Vigour	DW(%)	50mM NaCl(%)	100mM NaCl (%)	150mM NaCl (%)	200mM NaCl (%)
1	High	40	40	20	30	10
2	Medium	50	40	80	70	90
3	Low	10	20	0	0	0

Viability of seeds gradually deceased with successive increasing concentration of salt added to the petriplates.

Rate, Percentage and Index of Seed germination:-

The gradual decrease in % seed germination with increasing concentration of NaCl is evident from the present results where rate, percentage and index of germination declined with increasing salinity.





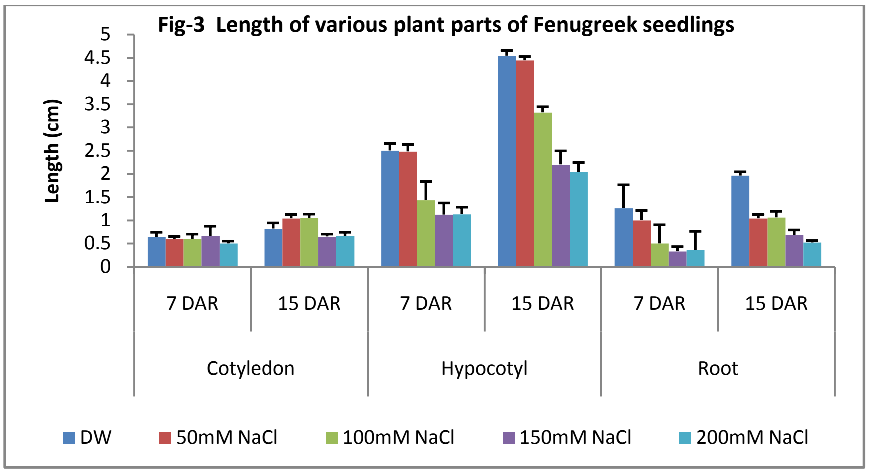
The results showed that increased NaCl significantly affected mean germination as compared to control treatment (Fig 1). Our studies also show that increasing levels of salt concentration were stressful especially 150mM and beyond, for germination. At the highest concentration of salt, germination was reduced to 60%. These observations are in conformity with those of Uniyal *et al*., (1998) according to him the effect of the saline conditions was more on the number of days to accomplish germination and percentage seedling emergence than percentage germination. Salts of different nature and concentration increase water potential, restricting the movement of water towards the seed surface (Tester and Devenport, 2003; Polesskaya *et al.,* 2006; Houimli *et al*., 2008). Under increasing salinity stress, water is not imbibed which delays the germination percentage (Almansouri *et al.*, 2001).

Seed germination is important stage of growth. Salinity inhibited the germination and germination rate of *Trigonella foenum-graecum* as the salt treatment increased. High salt stress is known to disturb osmotic and ionic homeostasis that limit the availability of water to the seeds and ultimately cause slow and poor germination. It is assumed that in addition to toxic effects of accumulation of high concentration of some ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (Maas and Nieman, 1978). So, it can be concluded that the delay in germination was mainly due to higher NaCl accumulation in the seeds; it appeared that NaCl adversely influenced fenugreek seedling characters. Factors adversely affecting seed germination may include sensitivity to drought and salt tolerance (Sadeghian *et al*., 2004). Salinity is a complex environmental constraint that presents 2 main components: an osmotic component due to the decrease in the external osmotic potential of the soil solution, and an ionic component linked to the accumulation of ions that become toxic at high concentrations (mainly Na, Cl, SO_4, CO_3, and HCO_3), and a stress-induced decrease in the content of essential elements, such as K and Ca. Some researchers have indicated that the main reason for germination failure was the inhibition of seed water uptake due to a high salt concentration, whereas others have suggested that germination was affected by salt toxicity (Mansour *et al*., 1994 and Khajeh-Hosseini *et al*., 2003).

Seedling Parameters:-

- **Shoot length (cm) and Root length (cm):-**

In all sets shoot length as well as root length both decreased with increasing salinity stress. High level of salinity has more drastic in reducing shoot length. In 200mM NaCl shoots showed stunted growth. The results showed that root and shoot length of *Trigonella foenum-graecum* at 50 and 100 mM were not significantly different from control treatment but 150 mM treatment was moderately and 200 mM NaCl treatment showed drastic difference from control. Growth and survival of plants at high salinity depends on adaptation to both low water potentials and high sodium concentrations, with high salinity in the external solution of plant cells producing a variety of negative concentrations.
Abiotic stresses are reported to alter levels of plant growth hormones leading to decrease in plant growth (Gupta *et al*., 1993). The shoot and root length are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress Jamil (2004).

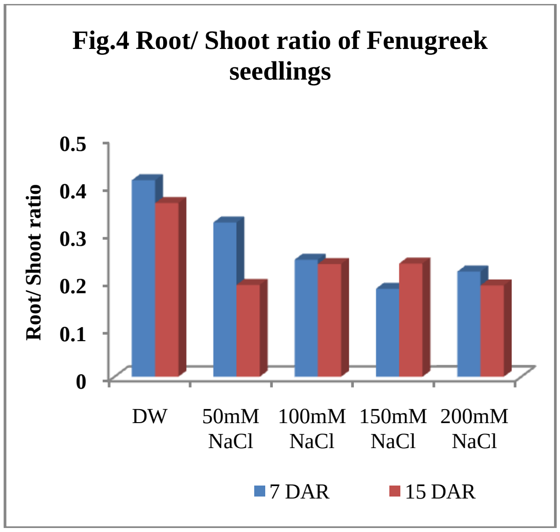


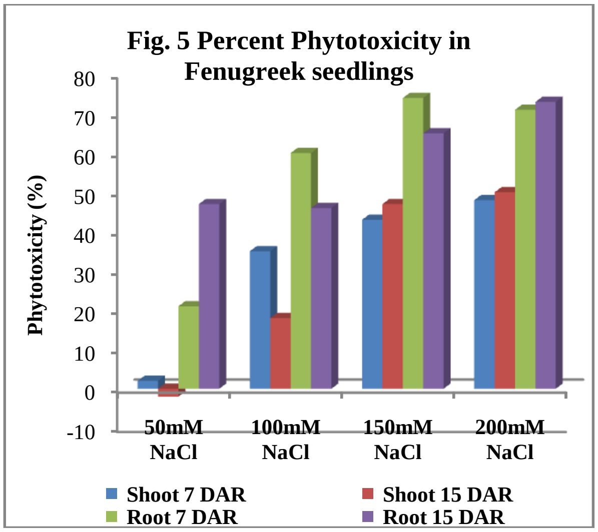
- **Fresh matter (g) and Dry matter (g):-**

With increase in salinity levels, both fresh weight and dry matter of seedlings declined. Salt stress causes ionic imbalance (Zhu *et al*., 1997), with excess sodium and chloride ions having a deleterious effect on many cellular systems (Serrano *et al*., 1999b), therefore plant survival and growth depends on adaptations to re-establish homeostasis. High salinity also inflicts hyper osmotic shock on plants, as chemical activity of water is decreased, causing a loss of cell turgor. Salt induced reduction in chloroplast stromal volume and generation of Reactive Oxygen Species (ROS) also plays an important role in decreasing plant photosynthetic capacity and therefore growth (Price and Hendry, 1991).

- **Root/Shoot ratio:-**

The root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress (Jamil and Rha, 2004). This ratio decreased with salinity significantly and the decrease was more pronounced at the highest level of stress. Salt stress inhibited the seedling growth (root and shoot length). Inhibition of plant growth by salinity may be due to the inhibitory effect of ions. The reduction in root and shoot development be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. It may be due to the ability of the root system to control entry of ions to the shoot is of crucial importance to plant survival in the presence of NaCl (Hajibagheri, 1989). High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Werner and Finkelstein, 1995) may be another reason for this decrease.



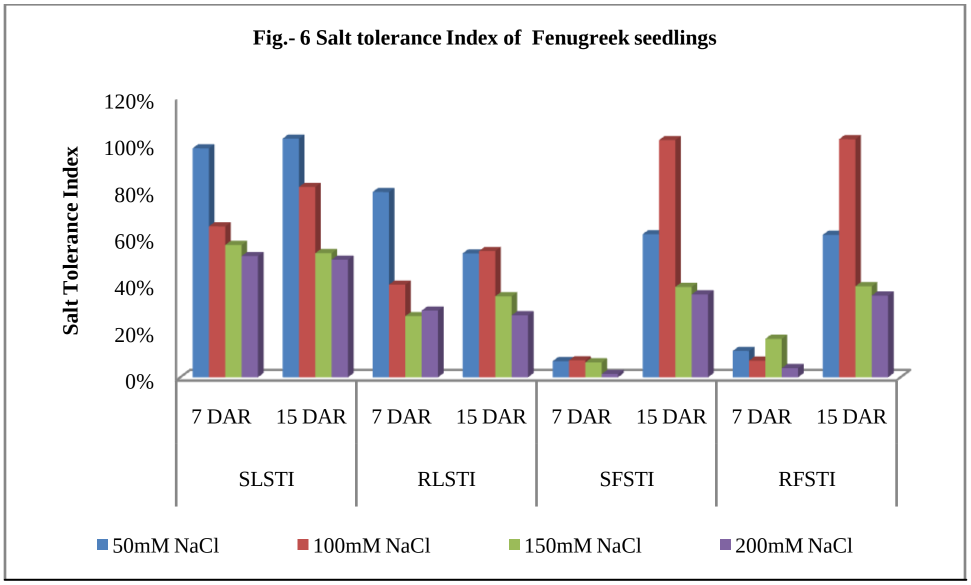


- **Percent Phytotoxicity:-**

Phytotoxicity of shoots as well as roots decreased at lower concentration and increased at higher concentration of NaCl used.

- **Tolerance index:-**

Similarly Salt Tolerance indices of roots as well as shoots decreased successively with increasing concentration of salt stress in the sets but interestingly shoots as well as roots of 100mM NaCl added sets showed an increase of fresh weight salt tolerance indices in15 Days old fenugreek seedlings.



Conclusion:-

Present study provides important information about the impacts of salinity on growth of Fenugreek seedlings. Results indicated that the effect of various salinity levels on seed germination was deleterious at highest level of NaCl. Salinity levels at 200 mM had significantly different effects on seed germination and growth. In total, seed germination rate, percent of germination, root and shoot lengths decreased with increasing salinity concentration. The control treatment showed there existed clear variation between different sets in terms of emergence percentage, fresh weight and dry weight of various plant parts. Basically, fresh weights decreased as shoot and root length declined after salinity levels increased. The inhibition was stronger in roots than in shoots. The increasing salt concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices and the seedling vigor indices. These methods and results thus provide a model system to screen for various concentrations of salt for their phytotoxic effects and also screen for the seeds able to counteract the deleterious effects of salt concentrations present in various types of irrigation waters and agricultural soils. Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to ability of seeds for best germination under unfavorable conditions, so necessity of evaluation of salt resistance plant species are important at primary growth stage. The findings suggest that fenugreek might tolerate moderate levels of salinity and can be tried for cultivation on marginal salted soils due to their tolerance to moderate salinity at germination and seedling stage. However, further study is needed to test their salt tolerance under field conditions to assess the possibility of cultivation of medicinal plants on otherwise unproductive lands.

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